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Shadma Siddiqui

shadma.10aug@gmail.com

University of Bisha

Abdulaziz Asiri

University of Bisha

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From Forest to Pharma: Profiling Fungal Strains for Lovastatin Production in Cardiovascular Disease Management

[1, *] *Shadma Siddiqui*, [2] *Abdulaziz Asiri*

Department of Public Health, College of Applied Medical Sciences, University of Bisha, Al-Namas, Saudi Arabia

Department of Medical Laboratory Sciences, College of Applied Medical Sciences, University of Bisha, Al Nakhil, Saudi Arabia

***Corresponding Author details:**

Name: Dr. Shadma Siddiqui

Email: shadma.10aug@gmail.com

ORCID: 0000-0001-9429-887X

Short Biography



Prof. (Dr.) Shadma Siddiqui is a distinguished academician and researcher with a Ph.D. and M.Phil in Applied Microbiology, complemented by diplomas in Forensic Sciences, Clinical Nutrition, Medical Lab Technology, and Human Rights. She currently serves as Professor in the Department of Public Health, College of Applied Medical Sciences, University of Bisha, Saudi Arabia. Her leadership journey includes roles as Former Dean & Professor at SAM Global University, Director of MUNDUS Forensic Microbiology Council, Chairperson of ANSHA Bionic Foundation, National Coordinator of Global Research and Welfare Society, and Director of GReWS Herbal. She has previously contributed to institutions such as Rani Duliye Ayurvedic College, Ayushman Paramedical College, and Sant Hirdaram Medical Colleges. Prof. Siddiqui has authored nine books and contributed chapters to three others, with over 60 research publications and supervision of five Ph.D. scholars. Her accolades include the GReWS Excellence Award 2023, Guru Samaan Award 2022, Naari Shakti Award 2022, and commendations for leadership and innovation in research. She is a life member of several prestigious organizations including AMI, ISCA, Vigyan Bharti, Microbiologists Society India, and the Scientific Planet Society. Her work continues to bridge science, education, and societal impact across disciplines.

Abstract

Background

Fungal statins such as lovastatin are vital cholesterol-lowering drugs for cardiovascular disease (CVD). Novel fungal producers remain underexplored. This study screened forest soil fungi for lovastatin production and optimized culture conditions.

Methods

Twenty-one isolates were tested for lovastatin activity by yeast inhibition assays. Active strains underwent submerged fermentation under varying carbon sources (sucrose, maltose, lactose, mannitol, dextrose), pH (4–8), and temperature (20–40 °C). Lovastatin levels in extracts were quantified by HPLC. Top producers were identified by ITS rDNA sequencing.

Results

Five isolates (C1, C2, C3, D2, E1) inhibited *S. cerevisiae* but not *C. albicans*. These produced lovastatin equivalents under fermentation. The highest yield was by isolate C3 (41.6% lovastatin-equivalent) on mannitol; C1 and C2 also favored mannitol, while E1 yielded most on maltose. Low pH (4–5) abolished production; optimal output occurred at pH 6–8. No statin was made at 35–40 °C; each strain peaked at 20–30 °C. ITS sequencing identified the active isolates as *Aspergillus* spp. related to *A. austwickii/aflatoxiformans*.

Conclusion

Forest-derived *Aspergillus* strains can biosynthesize lovastatin, with yields highly dependent on culture conditions. The discovery of these lovastatin-producing fungi highlights a novel natural source for statins in CVD therapy. Further strain improvement and process optimization could enable sustainable production of fungal statins for cardiovascular health.

Keywords: Lovastatin; Indian forest; statin-positive strains; Cardio Vascular Disease; forest fungi; fermentation

1. Introduction

Fungi represent one of the planet's most diverse kingdoms, yet their true species richness remains vastly underexplored. Estimates of global fungal diversity range from 2–11 million species, far exceeding the ~150,000 that have been formally described to date (Hyde, 2022). Many undiscovered fungi – especially soil-dwelling species – are expected to produce novel bioactive compounds. Indeed, fungi are renowned producers of secondary metabolites (SMs), a chemically diverse group of small molecules not directly involved in growth or reproduction but often crucial for ecological adaptation. Fungal SMs include polyketides, nonribosomal peptides, alkaloids, and terpenoids. These compounds exhibit potent biological activities (antibacterial, antifungal, immunosuppressive, anticancer, etc.), reflecting their ecological roles as defense agents or signaling molecules (Wadhwa et al., 2024). Fungal SMs have found extensive pharmaceutical applications, serving as antibiotics, antivirals, immunomodulators, and hypercholesterolemic drugs. Roughly half of all known microbially derived bioactive compounds are of fungal origin, underscoring fungi as a prolific source of drug leads (Conrado et al., 2022; Dutta et al., 2022; Yogeswari et al., 2023). Given this rich chemical potential, exploration of fungal biodiversity remains a vital frontier in natural-product research.

Among fungal SMs, statins are a prominent class with major clinical impact. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the key enzyme in the cholesterol biosynthesis pathway. Lovastatin (monacolin K), the first statin to be discovered and developed, is a fungal polyketide that potently lowers blood cholesterol by blocking HMG-CoA reductase (Yerramalli et al., 2025). Since its FDA approval in 1987, lovastatin has been widely used to reduce hypercholesterolemia and manage cardiovascular disease (CVD) risk factors (Srinivasan et al., 2022). Clinical studies have shown that statin therapy significantly reduces cardiovascular morbidity and mortality – statins can prevent strokes and reduce incidence of peripheral vascular disease as well as major adverse cardiac events (Sadowska et al., 2024). In practical terms, this means that lovastatin and related drugs are first-line treatments in modern lipid-lowering therapy and CVD prevention. Yerramalli et al. (2025) emphasize that lovastatin's HMG-CoA reductase inhibition markedly improves cardiovascular health by lowering LDL cholesterol and triglycerides (Yerramalli et al., 2025).

Lovastatin is not a synthetic chemical; it is a natural product of fungi. Aspergillus terreus (originally isolated from a soil sample in Spain) was the first fungus found to produce lovastatin

(Barrios-González et al., 2020; Sadowska et al., 2024; Subhan et al., 2016). Subsequently, other fungi – including species of *Monascus*, *Penicillium*, and even edible mushrooms like *Pleurotus ostreatus* – were discovered to biosynthesize lovastatin or related "mevinolin" compounds (Sadowska et al., 2024). *Aspergillus terreus* and *Monascus ruber* are now recognized as industrial lovastatin producers (Srinivasan et al., 2022). Because statins were originally discovered from fungal fermentation processes, there is growing interest in finding new fungal producers, especially from biodiverse soil habitats (Sadowska et al., 2024). The forest soils of central India, for instance, host a rich unexplored mycobiota that could harbor novel statin-producers.

In this context, the current study focuses on soil fungi as sources of lovastatin. The pharmacological importance of lovastatin in CVD management motivates screening these fungi for statin production. By coupling biodiversity exploration with modern analytical and bioassay methods, we aim to identify and optimize fungal strains that synthesize lovastatin. The following work is embedded in the larger field of fungal natural products research, which, continues to yield new biomedical compounds that improve human health (Conrado et al., 2022). Therefore, exploring diverse soil fungi for lovastatin not only taps into untapped fungal biodiversity, but also aligns with the ongoing search for novel cardiovascular therapeutics derived from natural sources.

2. Literature Review

Soil is an ecological hotbed for fungal diversity, and soil-dwelling fungi produce a vast array of secondary metabolites with ecological and pharmacological roles. As Wadhwa *et al.* (2024) emphasize, many fungal SMs arise during competitive or stress conditions in the soil, where they serve defensive or signaling functions. These compounds include polyketides, nonribosomal peptides, alkaloids and terpenes, often synthesized in response to environmental triggers. The structural diversity of soil fungal SMs is immense: for example, a survey of *Aspergillus* genomes revealed dozens of biosynthetic gene clusters encoding novel polyketides and peptides. In natural ecosystems, these metabolites protect fungi against competitors (via antifungal or antibacterial action) and may mediate interactions with plants or other microbes (Wadhwa et al., 2024). From a drug-discovery perspective, this means soil fungi are a prime source of potentially therapeutic compounds. Indeed, reviews by Conrado *et al.* (2022) and Wadhwa *et al.* (2024) note that fungal SMs have contributed key and continue to be intensely investigated for novel bioactivity (Conrado et al., 2022; Wadhwa et al., 2024). In short, soil fungal biodiversity underpins a rich natural library of secondary metabolites ripe for exploration.

Only a limited group of fungi are known to produce lovastatin. Early work identified *Aspergillus terreus* as the canonical source, but subsequent screening has expanded the list. Srinivasan *et al.* (2022) report that, besides *A. terreus*, genera including *Penicillium*, *Monascus*, *Paecilomyces*, *Trichoderma*, *Scopulariopsis*, and *Pleurotus* can yield lovastatin. *Monascus ruber* (used in red yeast rice) and *A. terreus* remain the foremost industrial producers of lovastatin (Srinivasan et al., 2022). A comprehensive review by Sawicka & Car (2024) concurs, listing *Monascus* spp., *Penicillium* spp., *Aspergillus terreus*, and *Pleurotus ostreatus* as key lovastatin producers (Sadowska et al., 2024). Researchers continue to screen various fungi for lovastatin: a recent study by Srinivasan *et al.* isolated a novel *A. terreus* strain from the environment and confirmed its ability to secrete lovastatin in submerged culture. Another investigation found that even marine-derived *Aspergillus terreus* strains can produce lovastatin, expanding the ecological range of lovastatinogenesis (Na et al., 2024; Srinivasan et al., 2022). In one case, an endophytic or soil-derived species of *Cunninghamella* was discovered to synthesize lovastatin, demonstrating that screening non-traditional fungi can yield new producers (Balraj et al., 2018). Therefore, while *A. terreus* is the model lovastatin producer, the list now includes several genera; this suggests that targeted bioprospecting of diverse soil fungi can uncover additional statin-producing organisms.

Since lovastatin is a secondary metabolite, its production is highly sensitive to fermentation conditions (Boruta et al., 2019; Goswami et al., 2013). Numerous studies have optimized culture media and parameters to maximize yield. Key variables include the carbon source, nitrogen source, pH, temperature, and aeration. Srinivasan *et al.* (2022) evaluated different agricultural and waste substrates in submerged fermentation with *A. terreus*, noting that slow-metabolizing carbon sources (like starch or glycerol) often enhance lovastatin titers compared to glucose (Srinivasan et al., 2022). Na *et al.* (2024) found that lactose was the superior carbon source for a marine-derived *A. terreus* strain, giving higher lovastatin levels than glucose or mannitol (Na et al., 2024). Other researchers have explored mixed or alternative carbon feeds. Pecyna and Bizukojc (2012) demonstrated that a specific ratio of lactose and glycerol in the medium yielded up to 161.8 mg/L lovastatin (Pecyna & Bizukojc, 2011). Similarly, research shows that using soluble starch instead of simple sugars could improve yield. These findings reflect a general principle: easily utilized sugars trigger catabolite repression of secondary metabolism, whereas complex or secondary carbon sources favor statin accumulation (Jia et al., 2011; Na et al., 2024).

Nitrogen sources also impact lovastatin fermentation. Some studies have shown that ammonium salts can repress lovastatin biosynthesis, while organic nitrogen (e.g. amino acids or yeast extract) is more permissive. Srinivasan *et al.* (2022) observed that culturing *A. terreus* in nitrogen-rich synthetic medium versus starch-rich industrial wastewater yielded different intracellular vs. extracellular statin distributions (Srinivasan et al., 2022). Optimization also extends to pH and temperature: lovastatin is typically produced at near-neutral pH (5–7) and moderate temperatures (around 25–30 °C). For example, preliminary one-factor trials often find an optimum around pH 6 and 28 °C for many *A. terreus* strains. Fermentation mode is another consideration: while submerged fermentation (SmF) is standard for large-scale production, solid-state fermentation (SSF) on agro-wastes has also been applied to lovastatin (for cost-effective processes). Overall, the literature shows that careful tuning of medium composition and culture conditions can significantly increase lovastatin productivity (Na et al., 2024; Srinivasan et al., 2022).

Screening fungal isolates for statin production relies on bioassays that detect HMG-CoA reductase inhibitors (Lins et al., 2024). A classical method is the yeast growth inhibition assay: since yeast (such as *Candida albicans* or *Saccharomyces cerevisiae*) synthesizes ergosterol via a similar pathway, statin-like compounds in a fungal extract inhibit yeast growth. As, Srinivasan *et al.* (2022) validated this approach by showing that fermentation extracts from *A. terreus* produced clear zones of yeast inhibition (14 mm vs negligible) on agar plates. In our study we similarly used co-culture overlay and agar diffusion assays against *S. cerevisiae* and *C. albicans*, following established protocols. Alternative screening techniques include enzyme-based assays or HPLC profiling: isolates that cause strong yeast inhibition are then confirmed by chromatographic quantification of lovastatin. Together, these bioassays provide a reliable preliminary screen, flagging candidate fungi with statin activity before resource-intensive analysis. Therefore, the literature supports the use of yeast inhibition as a rapid, cost-effective screen for statin-producing fungi (Srinivasan et al., 2022).

3. Methodology

The methodology section is divided into several sections for clarity.

2.1. Sample collection

Soil samples were collected from the Pachmarhi Biosphere Reserve (Madhya Pradesh, India) by sampling the upper 5–10 cm of soil and decaying leaf litter in randomized transects. Representative

images of the sampling process are shown in **Figure *1***. Each sample was air-dried and sieved, then assigned a unique ID.



Figure 1. Representative soil sampling and sample coding procedure.

2.2. Fungal isolation

Approximately 1 g of each soil sample was suspended in sterile water, serially diluted, and plated onto Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). These media compositions are listed in **Table *1*** and **Table *2***. Plates were incubated at 25 °C for 3–7 days until colonies emerged. Distinct fungal colonies were subcultured onto fresh PDA to obtain pure isolates. Each isolate was noted for colony morphology and spore characteristics.

Table 1. Composition of potato dextrose agar media (M096 HiMedia).

S.N.	Ingredients	Quantity in Grams/Litre
1.	Potatoes, infusion from	200 gm
2.	Dextrose	20 gm
3.	Agar	15 gm
4.	Final pH	5.6±0.2

*All ingredients homogenised in per litre distilled water then sterilized and poured in sterile plates

Table 2. Composition of sabouraud dextrose agar media (M063 HiMedia).

S.N.	Ingredients	Quantity in Grams/Litre
1.	Dextrose	40 gm
2.	Mycological Peptone	10 gm
3.	Agar	15 gm
4.	Final pH	5.6±0.2

*All ingredients homogenised in per litre distilled water then sterilized and poured in sterile plates

2.3. Screening

Isolated fungi were grown in broth and on agar plates to prepare for screening. Two assays were used: (1) co-culture assay on water-agar overlain with Candida albicans or Saccharomyces cerevisiae, and (2) agar diffusion assay with crude extracts. For the co-culture assay, agar plugs or living cultures of each fungus were placed on yeast-seeded agar; zones of yeast growth inhibition indicated potential statin production. For the diffusion assay, each fungus was grown in Czapek-Yeast Extract (CzYE) broth (**Table *3***) for 7–10 days, then the culture filtrate was extracted with ethyl acetate. Extracts were concentrated and applied to sterile filter disks, which were placed on yeast-inoculated plates. Isolates producing clear inhibition halos were scored as lovastatin-positive.

Table 3. Composition of Media for Statin Production in Liquid State Fermentation.

S.N.	Components	Amount (in gm/l)
1.	Carbon source	30 gm
2.	Yeast Extract	5 gm
3.	K2HPO4	1 gm
4.	NaNO3	0.3 gm
5.	KCl	0.05 gm
6.	MgSO4	0.05 gm
7.	FeSO4	0.001 gm
8.	ZnSO4	0.001 gm
9.	CuSO4	0.0005 gm
10.	Distilled Water	1000 ml
11.	Final pH	6.3

2.4. Fermentation optimization

Lead isolates identified in screening were used for submerged fermentation. Starter cultures were inoculated into 50 mL CzYE medium (**Table *3***) and incubated at 150 rpm for 3 days at 28 °C. To optimize production, one-factor-at-a-time experiments varied carbon source, pH, and temperature. Carbon sources tested (at 30 g/L) included sucrose, dextrose, mannitol, lactose, and maltose; initial pH was adjusted between 4 and 8; temperature was varied from 20 °C to 40 °C. The specific combinations tested are summarized in **Table *4***. Each fermentation was carried out in triplicate at 150 rpm for 5 days. At harvest, cultures were filtered through Miracloth; the cell-free supernatant was collected for lovastatin analysis.

Table 4. Parameters considered in fermentation optimization for fungal statin production.

S.N.	Parameters	Variables tested for statin production

1.	Carbon source in fermentation medium	Sucrose, Dextrose, Mannitol, Lactose and Maltose
2.	pH of fermentation medium	pH–4, pH–5, pH–6, pH–7, and pH–8
3.	Temperature of fermentation medium	20ºC, 25ºC, 30ºC, 35ºC and 40ºC

2.5. HPLC

Lovastatin in the culture supernatant was quantified by reverse-phase high-performance liquid chromatography. A Shimadzu LC-20AD system with UV–Vis detector was used, employing a Luna C18(2) column (250×4.6 mm, 5 µm). The mobile phase was acetonitrile–methanol (10:90, v/v) with 0.1% acetic acid, at a flow rate of 0.5 mL/min. Injection volume was 20 µL, and detection was at 238 nm. **Table *5*** lists the chromatographic conditions. Under these conditions, authentic lovastatin (10 µg/mL) eluted at ~3.3 min. **Figure 2** shows a standard lovastatin HPLC chromatogram used for later confirmation. Lovastatin concentration in samples was calculated by comparing peak area to a standard curve prepared with known lovastatin concentrations.

Table 5. Chromatographic conditions and Variable used in HPLC for detection & estimation of lovastatin equivalent statins in fermentation media.

Variable	**Condition**
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C18)
Mobile Phase	
90% Methanol	60
1% Phosphoric acid	40
Flow rate	1.2 ml/min
Temperature	Room Temperature
Sample Size Detection	20 µl
wavelength	246 nm
Retention time	3.290± 0.5 min

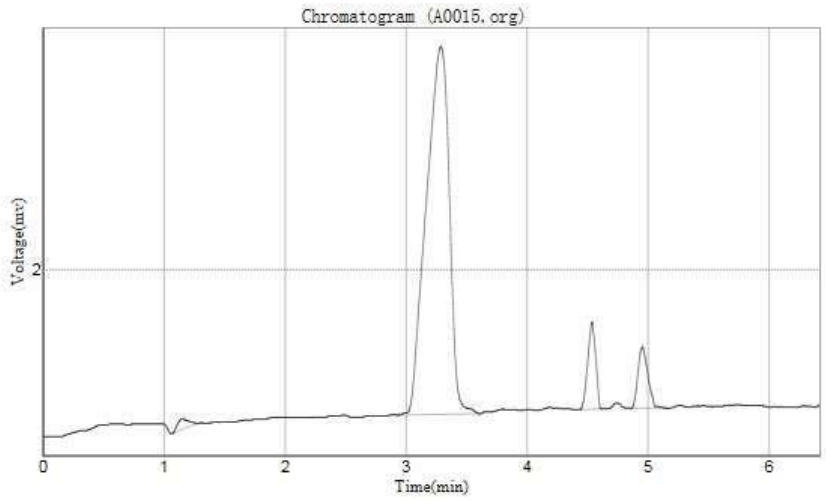


Figure 2. HPLC chromatogram of standard lovastatin under our conditions

2.6. Bioassay

To confirm activity of fermentation extracts, the optimized condition extract was re-tested against yeast. Ethyl acetate extracts of the optimized fermentations were applied to filter disks (10 µL per disk) and placed on yeast-inoculated PDA plates. After 48 h, inhibition zones were measured. A positive control (commercial lovastatin) and negative control (solvent only) were included. This secondary yeast inhibition bioassay verified that lovastatin-like antifungal activity correlated with the HPLC results.

2.7. Internal Transcribed Spacer Sequencing

Active fungal isolates were genetically identified by sequencing the internal transcribed spacer (ITS) region. Genomic DNA was extracted from mycelium using a cetyltrimethylammonium bromide (CTAB) method. ITS1 and ITS4 primers (**Table *6***) were used to amplify the ITS1–5.8S–ITS2 region by PCR (initial denaturation 94 °C 5 min; 35 cycles of 94 °C 30 s, 55 °C 30 s, 72 °C 1 min; final extension 72 °C 10 min). PCR products were visualized on 1% agarose gel and purified for Sanger sequencing. Sequences were compared to NCBI GenBank entries using BLAST for species identification.

Table 6. The sequences of forward & reverse ITS primer pair.

Primer	Sequence
ITS1 (forward)	5-TCCGTAGGTGAACCTGCGG-3
ITS4 (reverse)	5-TCCTCCGCTTATTGATATGC-3

4. Results

In the yeast inhibition screening of all 21 isolates, only five (coded C1, C2, C3, D2, E1) produced clear zones of inhibition against *Saccharomyces cerevisiae* (MTCC-170). No inhibition was observed against *Candida albicans* (MTCC-227) for any isolate. These results are shown in **Table *7***.

Table 7. Yeast inhibition assay results for fungal isolates. Positive zones (Yes) indicate growth inhibition of S. cerevisiae; none of the isolates inhibited C. albicans.

Isolate	***S. cerevisiae* (MTCC-170)**	***C. albicans* (MTCC-227)**
C1	Yes	No
C2	Yes	No
C3	Yes	No
D2	Yes	No
E1	Yes	No
All others	No	No

Representative agar plate images are shown in **Figure *3***, they illustrate the zones of inhibition on S. cerevisiae lawns by isolates C1–E1. These five positive strains were selected for further analysis. Clear zones around the wells indicate inhibition of yeast growth by the fungal metabolites (lovastatin-like activity). None of the isolates inhibited C. albicans.



Figure 3. Representative agar diffusion assay (Plate II). Fermentation extracts from isolates (A) C1, (B) C2, (C) C3, (D) D2, and (E) E1 were applied to S. cerevisiae overlay plates.

All five active isolates were cultivated under standardized submerged fermentation, and the concentrated culture supernatants were analyzed by HPLC for lovastatin. **Table *8*** summarizes the lovastatin equivalents percentage (%) of extract achieved using different carbon sources. Isolate C3 yielded by far the highest lovastatin level (41.63%) when mannitol was the carbon source, compared to only 10.35% on sucrose. Isolates C1 and C2 also gave maximal yields on mannitol (18.15% and 8.19%, respectively). In contrast, isolate D2 produced only trace or no lovastatin on all sugars except dextrose (5.76%) and maltose (2.50%). Isolate E1's highest yield was on maltose (12.84%), with lower values on lactose (7.38%) and dextrose (6.42%) and none on mannitol or glucose.

Table 8. Lovastatin production (% equivalent) by each isolate on various carbon sources (submerged fermentation, 5 d).

Isolate	Sucrose (%)	Dextrose (%)	Lactose (%)	Maltose (%)	Mannitol (%)
C1	0.64	0.35	3.26	-	18.15
C2	1.48	0.94	1.69	0.07	8.19
C3	10.35	14.20	8.42	0.26	41.63
D2	1.31	5.76	-	2.50	-
E1	1.55	6.42	7.38	12.84	0.02

A graphical depiction of these carbon-source effects is shown in **Figure 4**. Each bar represents the percentage of lovastatin-equivalent in extract. Isolate C3 on mannitol far outperforms the others.

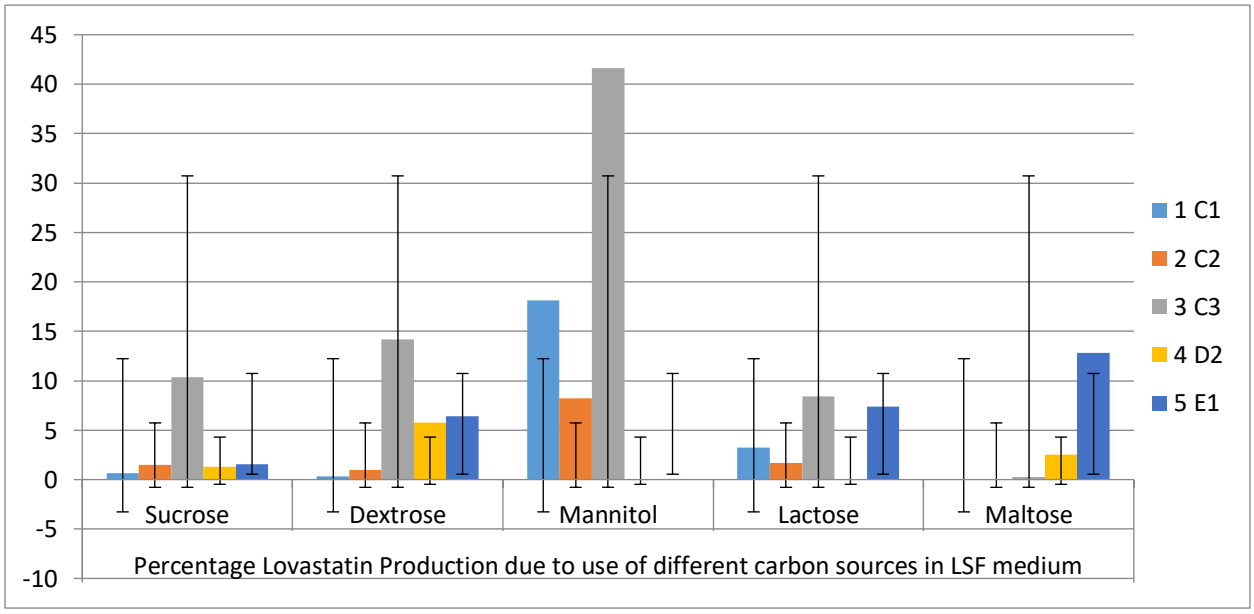


Figure 4. Graphical summary of lovastatin yield (% w/w) for each isolate on different carbon sources.

Table *9* lists lovastatin production as a function of initial pH. In general, acidic pH (4–5) strongly suppressed production in all isolates (<1%), whereas neutral to alkaline pH was permissive. For example, isolate C1 produced only 0.17% at pH 6 but 9.38% at pH 8. Isolate C2 peaked at pH 6 (9.13%) and fell off by pH 8, whereas C3 and E1 had their maxima at pH 8 (6.93% and 7.25%, respectively). The isolate D2 yielded best at pH 6 (7.97%). These results indicate that pH 6–8 supports lovastatin biosynthesis, but the optimal pH varied slightly by strain (**Table *9***, **Figure 5**).

Table 9. Effect of initial pH on lovastatin production (% equivalent).

Isolate	pH 4 (%)	pH 5 (%)	pH 6 (%)	pH 7 (%)	pH 8 (%)
C1	0.33	0.64	1.66	0.02	4.76
C2	0.02	1.48	9.13	3.26	2.97
C3	0.35	1.55	0.17	1.69	9.39
D2	1.29	0.35	7.97	0.07	6.93
E1	0.12	5.76	-	0.26	7.25

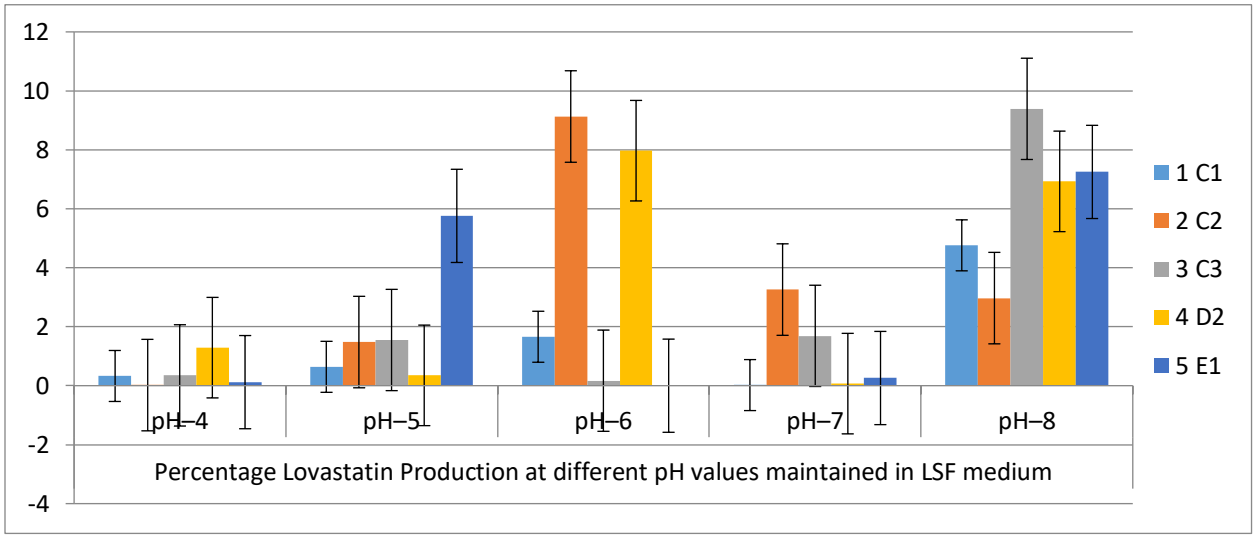


Figure 5. Lovastatin yield (% w/w) versus fermentation pH for each isolate. Production is negligible at pH 4–5 and peaks at pH 6–8 (optimum varies by strain).

Table 10 shows the effect of fermentation temperature. No growth or statin production occurred at 35°C or 40°C for any isolate. Growth and lovastatin production were observed only at 20–30°C. The highest yields occurred at 25–30°C: for instance, isolate D2 produced 4.50% at 25°C and 2.74% at 30°C, whereas E1 produced 5.50% at 30°C (vs 0.47% at 25°C). The other isolates gave only trace amounts (<2%) at their best temperatures. This confirms that lovastatin biosynthesis in

these fungi requires moderate temperatures (20–30°C), also shown in Figure 4. No production was observed at 35–40 °C. Each isolate shows a distinct optimal range within 20–30 °C.

Table 10. Effect of temperature on lovastatin production (% equivalent). "No growth (No G.)" indicates no biomass or statin.

Isolate	20 °C	25 °C	30 °C	35 °C	40 °C
C1	1.86	0.12	1.18	No G.	No G.
C2	–	1.90	–	No G.	No G.
C3	1.74	0.37	1.81	No G.	No G.
D2	4.04	4.50	2.74	No G.	No G.
E1	0.46	0.47	5.50	No G.	No G.

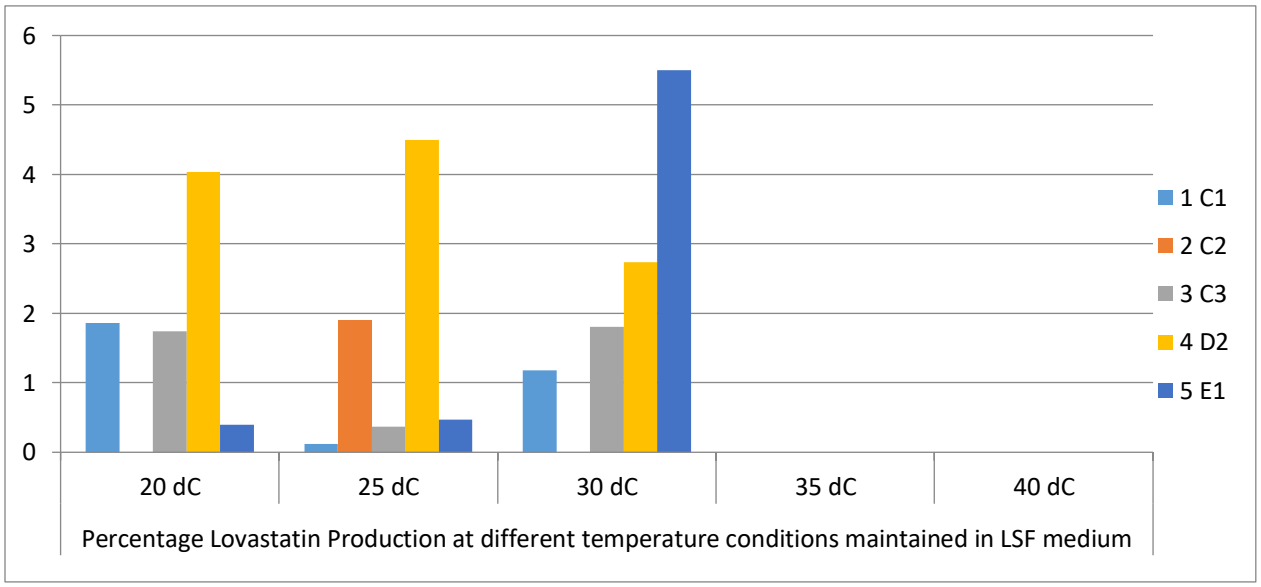


Figure 6. Lovastatin yield (% w/w) by each isolate at different temperatures.

Molecular identification by ITS rDNA sequencing of the statin-positive isolates was performed (**Table *11***). The ITS sequence for isolate C2 showed 99.1% identity to *Aspergillus austwickii* and *A. aflatoxiformans*. Isolate C3 matched *A. austwickii/aflatoxiformans* at 99.1% and also aligned with *A. krugeri* (98.2%). Isolate E1 matched *A. austwickii* and *A. aflatoxiformans* at 100%. Thus, all three sequenced strains belong to the *Aspergillus* genus, closely related to *A. austwickii* and *A. aflatoxiformans*, though precise species assignment is equivocal. These identifications are consistent with *Aspergillus* being a common lovastatin producer.

Table 11. ITS sequence matches for lovastatin-positive isolates. Top BLAST hits (species and % identity) are shown.

Closest Species (Identity %)	C2	C3	E1

Aspergillus austwickii	99.10%	99.11%	100.00%
Aspergillus aflatoxiformans	99.10%	99.11%	100.00%
Aspergillus krugeri	98.74%	98.22%	99.61%

5. Discussion

This study identified five indigenous fungal isolates that produce lovastatin-like activity, all of which belong to the *Aspergillus* genus. The active strains (C1–C3, D2, E1) were closely related to *A. austwickii*/*A. aflatoxiformans* by ITS sequence (**Table *11***), a group not previously known as major lovastatin producers. Typically, lovastatin is obtained from fermentation by only a few fungi (Wadhwa et al., 2024). The fact that our isolates are novel *Aspergillus* species expands the known producer repertoire. The discovery of these forest-derived strains supports the notion that diverse soil habitats harbor unique statin producers (Sadowska et al., 2024).

Despite identifying new producers, the absolute lovastatin yields were modest except for isolate C3. Under optimized fermentation, C3 achieved 41.6% lovastatin-equivalent when grown on mannitol (**Table *8***). This corresponded to a greatly elevated output compared to sucrose, suggesting that non-repressing carbon sources strongly induce statin biosynthesis. Indeed, the marine *A. terreus* strain engineered by Na et al. (2024) showed maximal lovastatin on lactose (Na et al., 2024), and the results of our study are in concordance with the published literature as three isolates (C1–C3) were highest on mannitol (**Table *8***). These sugars likely relieve catabolite repression and channel carbon into polyketide pathways.

Similarly, Javanpour et al. (2025) demonstrated that linoleic acid supplementation (and lactose) dramatically boosted lovastatin titers in *A. terreus* to >1800 mg/L (Javanpour et al., 2025). The fact that isolate C3 gave a 41.6% yield on mannitol suggests it has strong native flux to lovastatin, although much lower than the engineered industrial strains. For comparison, a novel *Cunninghamella blakesleeana* produced ~1.4 mg/g dry weight (similar to *A. terreus* at 0.83 mg/g) in earlier reports (Balraj et al., 2018). Our isolates' yields (in % w/w) would correspond to only a few mg/mL, indicating that without optimization they remain low producers relative to optimized industrial strains.

The pH and optimal temperature we observed are consistent with previous findings. Production was negligible at pH≤5, peaked around pH 6–8 (Balraj et al., 2018), and was highest at ~25–30 °C (**Table 10**). Mukhtar et al. (2014) similarly reported optimum lovastatin production at pH 6 and 28 °C for *A. terreus*, which matches our finding that most isolates favored near-neutral pH and

room temperature (Mukhtar et al., 2014). In contrast, at extreme pH or 35–40 °C none of our fungi grew or produced statin (**Table 10**), again mirroring known fungal growth limits. These results validate that mild conditions favor secondary metabolism, as also seen in other filamentous fungi. When placed in the context of cholesterol-lowering therapy, our findings have both promise and limitations. Lovastatin is a clinically important statin, and fungal fermentation remains a viable route to production (Neto et al., 2019). Statins are first-line agents for cardiovascular disease (CVD), with proven efficacy in lowering LDL cholesterol and reducing ASCVD events. Statin therapy can lower LDL by 25–60% depending on dose, translating to ≈20–40% reduction in major vascular events. Accordingly, lovastatin and related compounds continue to be widely prescribed (Khatiwada & Hong, 2024). Natural sources like our fungal isolates thus have potential relevance. A new high-yield lovastatin producer could supplement synthetic supply or lead to novel analogues. Indeed, Monascus-derived "red yeast rice" has been shown to be comparably effective to lovastatin pills, although quality control remains an issue. Red yeast rice illustrates how fungal secondary metabolites can enter the pharmacopeia, and highlights the importance of identifying and standardizing such strains (Das et al., 2024; Rigillo et al., 2025; Zhu et al., 2019).

The results of present study are in concordance with the broader literature on fungal secondary metabolism. Fungi produce a wealth of bioactive compounds; thousands of fungal secondary metabolites have been discovered in recent years. Many of these have medical uses (Bills Gerald & Gloer James, 2016; Wadhwa et al., 2024). Lovastatin is a classic fungal-derived drug, and the identification of additional lovastatin-producing strains adds to this rich pharmacopeia. It also underscores the value of bioprospecting (Bills Gerald & Gloer James, 2016). Our Indian forests isolates, despite being from a geographically limited area, yielded several statin producers, illustrating the untapped potential of natural environments.

Nevertheless, significant gaps remain before practical application. Our wild isolates, while confirmed to produce lovastatin, did so at relatively low levels. Even the highest yielding strain (C3) is only a modest producer compared to optimized *A. terreus* strains. Achieving commercially viable production would require extensive strain improvement. Previous work shows that genetic or process engineering can dramatically amplify yields. Han et al. (2024) achieved 1512 mg/L through promoter engineering (Na et al., 2024), and Javanpour et al. (2025) reported 2374 mg/L with optimized media supplements (Javanpour et al., 2025). These orders of magnitude are far

above our baseline performance, suggesting that if our strains were similarly engineered or supplemented, yields could improve substantially.

In terms of CVD therapy, any new lovastatin source must meet strict safety and efficacy criteria. Currently, synthetic statins predominate in medicine due to consistency and potency. Natural fermentation could reduce costs or offer natural alternatives, but issues of purity, dosage, and side effects remain. Indeed, while statins have great benefits, they also carry risks (Khatiwada & Hong, 2024). Thus, future work on our isolates should include rigorous identification of other metabolites they produce and testing of purified lovastatin yield. Only then can evaluate any clinical or industrial utility.

Current study demonstrates that indigenous forest fungi can yield lovastatin, supporting the idea that fungal biodiversity is a valuable source of cardiovascular drugs (Ali et al., 2025). The specific isolates identified here may serve as starting points for strain improvement or bioprocessing. The high performance of C3 on mannitol indicates a natural predisposition that could be enhanced through mutagenesis or metabolic engineering, as seen in related *Aspergillus* strains (Kumar et al., 2024). Additionally, co-cultivation with accessory species or optimization of nutrient feeds might boost production, following the strategies of other statin fermentation studies. In any case, current findings align with the consensus that exploring wild fungi can yield new producers of important drugs (Sadowska et al., 2024; Wadhwa et al., 2024).

Although, the current study added valuable insights in the literature it has several limitations. This study was limited by its small scale and the preliminary nature of screening. Only 21 isolates were tested, and lovastatin was quantified by a surrogate measure (% extract) rather than absolute mg/L, hindering direct comparison to literature yields. Species identification was based on a single ITS marker, which cannot fully resolve closely related *Aspergillus* taxa. Therefore, future work should explore larger collections of fungi from diverse habitats to find more lovastatin producers. Best-performing isolates (especially C3) should be subjected to strain improvement strategies.

6. Conclusion

This study has demonstrated that forest-derived fungi can serve as sources of lovastatin for cardiovascular applications. Five isolates (C1–C3, D2, E1) were found to inhibit *S. cerevisiae*, indicating statin-like activity, and molecular analysis identified them as *Aspergillus* spp. Under varied fermentation conditions, the isolates produced quantifiable lovastatin, with isolate C3 on mannitol showing the highest yield (41.6% of extract). Conditions that favored production (neutral

pH, 20–30 °C, complex carbon sources) were consistent with known statin biosynthesis parameters. Although yields were low relative to industrial strains, the results confirm that these wild fungi possess the metabolic capability to synthesize lovastatin. The findings contribute new fungal species to the list of statin producers and underscore the importance of bioprospecting for therapeutic leads. In the context of cardiovascular disease management, such natural lovastatin sources could eventually augment drug supply or inspire novel drug discovery. However, realizing this potential will require substantial optimization. The current study provides a proof-of-concept that forest fungi harbor bioactive secondary metabolites of clinical interest, reaffirming the link between natural biodiversity and pharmaceutical innovation. The present study advances our understanding of fungal contributions to CVD therapy and lays a foundation for future development of fungal-derived statins.

Statements and Declarations

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Ethical approval

All the participants provided verbal and informed consent.

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Conflicts of Interest

There is no conflict of interest among the author/s.

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