Comparative Evaluation of Agro-Industrial Substrates for Enhanced Lovastatin Production Using Solid-State Fermentation

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Abstract- The present investigation aimed to produce lovastatin using *Penicillium citrinum* isolated from soil through solid-state fermentation (SSF) employing agro-industrial waste substrates. Soil samples were processed using serial dilution, and fungal colonies were cultured and morphologically identified. Five solid substrates coconut oil cake, sugarcane bagasse, groundnut oil cake, groundnut shell, and rice husk were tested for their suitability to support fungal growth and metabolite production. Supplementation with carbon and nitrogen sources, including ammonium sulfate, yeast extract, and lactose monohydrate, was performed to enhance fermentation efficiency. After a 20-day incubation period, visible fungal growth was observed on coconut oil cake, sugarcane bagasse, and rice husk. The highest colony development was found on coconut oil cake, followed by sugarcane bagasse and rice husk. The spore count was highest in coconut oil cake (10^{10} spores/ml), followed by sugarcane bagasse (10^{8} spores/ml), and lowest in rice husk (10^{4} spores/ml). Approximately 5 grams of each fermented sample was extracted with 95% ethanol and subjected to centrifugation at 1500 rpm for 20 minutes. The resulting supernatant was tested for the presence of lovastatin using Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectral analysis confirmed the presence of characteristic functional groups of lovastatin, such as hydroxyl (–OH) and lactone (C=O), particularly in the coconut oil cake sample, which exhibited the most intense peaks. Sugarcane bagasse showed moderate peak intensity, while rice husk showed the least. The study concludes that Penicillium citrinum effectively produces lovastatin under SSF conditions, with coconut oil cake being the most promising substrate for maximum yield.

Keywords: Lovastatin, *Penicillium citrinum*, Solid-state fermentation, FTIR analysis, Agro-industrial waste.

INTRODUCTION

Lovastatin is a statin produced as a secondary metabolite from fungi and applied to lower blood cholesterol via inhibition of the enzyme HMG-CoA reductase. Given the rise in cardiovascular diseases, natural and cost-effective production of statins is a growing priority. Lovastatin is traditionally produced with *Aspergillus terreus*; however, as we demonstrate here, fungi- especially from the Penicillium genus- possess great potential as alternative sources of lovastatin. Microorganisms, such as fungi found in soil, have a wide array of metabolic potential and the ability to produce useful bioactive compounds. We isolated soil fungi and screened their ability to produce lovastatin, including *Penicillium citrinum*, shown to be a promising producer of lovastatin. The specific production method we used was solid-state fermentation (SSF), using agricultural waste products including, rice bran, sugarcane bagasse, and coconut oil cake as production substrates. The use of these waste products can contribute to low production costs while containing ample nutrients for fungal growth and metabolite production. Overall, our work shows the potential of soil fungi and agricultural waste as a sustainable and eco-friendly platform for the production of important pharmaceutical compounds such as lovastatin.

Lovastatin, a secondary metabolite produced by fungi, is a first-line drug in the treatment of hypercholesterolemia due to its potent inhibition of HMG-CoA reductase, the rate limiting enzyme of the mevalonate pathway [1][2]. Lovastatin is a lactone prodrug that is enzymatically hydrolyzed in vivo to the

active β-hydroxy acid moiety that competitively inhibits HMG-CoA reductase with a Ki of ~0.6 Nm [3]. While lovastatin is well known for reducing levels of cholesterol, lovastatin has pleiotropic effects unrelated to cholesterol, for example, lovastatin has been demonstrated to have anti-inflammatory properties by inhibiting NF-κB and anti-cancer properties by inhibiting prenylation of Ras proteins [4][5]. There is a growing body of evidence that lovastatin can be neuroprotective in Alzheimer's disease by reducing the aggregation of amyloid-β [6] and in fragile X syndrome by affecting Rho GTPases [7] Compared to red yeast rice extracts, which contain fluctuating levels of monacolin K and potential contamination with citrinin (up to 100 ppb in commercial products [8], lovastatin manufactured for pharmaceutical use provides consistency for dosing and safety. While the clinical importance of lovastatin is clear, lovastatin production at the industrial level faces some significant bottlenecks for the production of lovastatin. Endemics *Aspergillus terreus* strains in submerged fermentation (SmF) produce 100–300 mg/L of lovastatin, but glucose limits titers with carbon catabolite repression after 72 hours [9]. The production of fungal pellets that are free from shear stress is limited in stirred tank bioreactors, and when placed in sufficient shear from a stirred tank bioreactor can reduce productivity by 20–30% and reduce granularity of the pellet [10]. These bottle necks since downstream purification is complicated due to their co-production of (+)-geodin and other polyketides. The purification of lovastatin would require a very sophisticated purification on a chromatographic column, which could cost tens of thousands, if not a hundred thousand dollars [11]. One significant temperature factor in the production of lovastatin is temperature sensitivity. If there are deviations greater than 28±1 °C, lovastatin yield will be reduced by 15–20% from the action of the lovE transcriptional regulator which regulates, in some way, lovastatin biosynthesis [12]. Recently, recombinant yeast systems have been developed (i.e., in *Pichia pastoris*) to produce lovastatin. Although some promising results have been observed: the use of methanol-inducible promoters resulted in 180 mg/L, the proteolytic nature of heterologous enzymes continues to be a limiting factor in the production of lovastatin from these recombinant strains [13].

SSF provides a low-cost high efficiency alternative process to submerged fermentations (SmF), especially with agro-industrial residues as substrates. Rice bran, a byproduct rich in phytosterols, improved lovastatin production by 40%, compared to production in criterion (typical growth media) media, through the induction of the polyketide synthase (PKS) genes [14]. Sugarcane bagasse, an extremely porous substrate (85% void space) facilitates oxygen diffusion, thereby reducing energy consumption by 30% [15]. Careful optimization of moisture content (~60 - 70%) was required to prevent sporulation and maintained hyphal extension rates of 0.5 - 0.7 mm/h [16]. Blending substrates (e.g. wheat bran + coconut oil cake) also contributed to high production rates (~450 mg/L). For example, 7day SSF fermentation with wheat bran and coconut oil cake yielded > 450 mg/L lovastatin, which was attributed to activation of PKS by lipids [37]. Life cycle assessments show the environmental benefits for SSF as they demonstrated up to a 60% decrease in water usage, with up to 45% less CO_2 than SmF [17].

Genetic engineering recently, using CRISPR-Cas9 editing on the lovastatin biosynthetic gene cluster (lovB/lovF) in *A. terreus*, increased titers significantly (2.3-fold) by removing non ribosomal peptide synthase (NRPS) bottlenecks [18]. Similarly, one group overexpressed the transcription factor lovE, under gpdA promoter control, which increased flux through the polyketide pathway resulting in substantially improved product yields at 800 mg/L in optimized bioreactors [19]. Furthermore, they found that by using adaptive laboratory evolution (ALE) under lovastatin pressure, strains could be selected for enhanced acetyl-CoA carboxylase (ACC) activity (the precursors used to supply the polyketide pathway [20]. Co-culturing *A. terreus* with *Saccharomyces cerevisiae* increased pH stability to around 6.0–6.5 and extended the productive phase by about 24 h. In addition, methylotrophic yeasts (e.g*., Pichia pastoris*), which enable production via methanol-fed fermentation (180 mg/L), include some protease-deficient strains to minimize LovB degradation [21].

Metagenomic exploration of Antarctic soil fungi skewered isolates, one of which, *Penicillium crustosum,* produced a maximum of 120 mg/L lovastatin at 10°C, indicating the potential for cold-adapting PKS enzymes [22]. Strains of Fusarium from

marine sources are osmotic-stress tolerant (3.5% NaCl), and production yields were shown to increase by 35% under osmotic stress [23]. In addition, endophytic fungi (*Xylaria sp*.) were isolated from Catharanthus roseus, which co-produced lovastatin, as well as vinca alkaloids, for the potential dual-drug extraction of drugs [24]. As the authors mention, the future appears bright with developments such as design off new strains by AI to guide targets for PKS mutations from AlphaFold predictions, and microfluidic SSF bioreactors to track metabolite production in real-time [25]. Even so, a combination of new regulatory aspects, such as cGMP-package adaptation for product produced by SSF technology has followed behind the equally as rewarding approach of SmF technology despite being 30% cheaper [26].

MATERIALS AND METHODS

Isolation of Microorganism

The fungal isolate containing the fungi *Penicillium citrinum* was isolated from soil samples from cultivated fields in Gudalur, Tamil Nadu. We used the serial dilution method on Potato Dextrose Agar (PDA) to isolate the fungus which was then identified through colony morphology and micro morphological characteristics with a Lactophenol Cotton Blue (LPCB) stain; the isolate was stored on PDA slants in a refrigerator at 4 ± 1 °C when needed. [27].

Substrates

Agro-industrial residues such as coconut oil cake, sugarcane bagasse, rice bran, groundnut shell, and groundnut oil cake, were chosen as solid substrates. The substrates were collected from local agricultural processing units shade dried, powdered, and sieved to uniform particle size for fermentation [28].

Fig.1 Five substrates

Chemicals and Reagents

chemicals and media for this paper contained (PDA Media, Ethanol, 0.4g of Lactose monohydrate, 0.2g of Ammonium sulfate, 0.2g of yeast extract, Ferric chloride, Hydrochloric acid, and LPCB stain all chemicals and media were collected from the stock in the Department of Microbiology, JSS AHER, Ooty.[29].

Equipment

The main equipment used in the study were: autoclave, incubator, rotary shaker, centrifuge, colorimeter and a FTIR Spectrophotometer [30].

Isolation and Identification of Fungal Strain

Soil samples were subjected to serial dilution to 10^{-5} and 0.1 mL from each dilution was spread on PDA plates. Exposed to incubate at 28 ± 2 °C for 5–7 days to allow for fungal growth.[38] Separated morphologically different colonies and each so-called fungus was sub-cultured and based on morphology and microscopic observation using Lactophenol cotton blue (LPCB) staining identified as *Penicillium citrinum* [31].

Preparation of substrates

Weigh 10 g of each agro-substrate in separate conical flasks of 250 ml. Add sterile distilled water to obtain approximately 65% moisture content. All flasks contained 0.4 g lactose monohydrate (as carbon source), 0.2 g ammonium sulfate, and 0.2 g yeast extract (as nitrogen sources) to support fungal growth and lovastatin production. All flasks were sterilized

by autoclaving 121 °C for 15 minutes [28].

Preparation of Inoculum

A spore suspension was prepared for *Penicillium citrinum* by adding 10 mL sterile distilled water with 0.1% Tween 80 to a 7-day-old PDA slant. Excess water was carefully scraped off with a sterile loop, the spores being filtered through sterile cotton to remove any remaining mycelial debris [31].

Solid-State Fermentation (SSF)

Each sterilized substrate flask was inoculated with 2 mL of freshly prepared fungal spore suspension under aseptic conditions. The flasks were left to incubate at 28 ± 2 °C for 20 days under static conditions. Fungal growth and colonization of substrates were visually observed.[32].

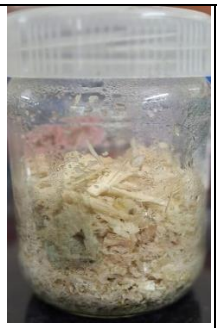
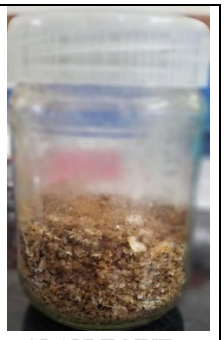
Before Fermentation	 RICE HUSK	 SUGARCANE BAGASSE	 COCONUT OIL CAKE	 GROUNDNUT OIL CAKE	 GROUNDNUT SHELL
After Fermentation	 FUNGAL GROWTH IN RICE HUSK	 FUNGAL GROWTH IN SUGARCANE BAGASSE	 FUNGAL GROWTH IN COCONUT OIL CAKE	 NO FUNGAL GROWTH IN GROUNDNUT OIL CAKE	 NO FUNGAL GROWTH IN GROUNDNUT SHELL

Table 1: FERMENTED SUBSTRATES

Lovastatin Extraction

The fermented substrates were dried and ground into finer particles after fermentation. Five grams of each sample was combined with 50 mL of ethanol (ethanol-1, general purpose) and shaken for 2 hours using a rotary shaker (150 rpm). The samples were centrifuged for 10 minutes at 5000 rpm (Eppendorf 5415 C) and the supernatant was collected for further analysis [32].

Lovastatin Colorimetric Detection

The colorimetric estimation was performed using the protocol of Valera *et al.* (2005). One ml of the ethanolic extract was combined with 1.0 ml of ferric chloride reagent (1% FeCl3 in 0.1 N HCl) and allowed to stand at room temperature for 10 minutes. A reddish-brown color developed after standing for 10 minutes which was read, at 530 nm, using a colorimeter. The Interest in the concentration of lovastatin, and therefore, compared the absorbance values to a standard curve prepared using pure lovastatin [33].

RESULT

Identification of *Penicillium citrinum*

Soil samples were obtained from Gudalur and assessed using the serial dilution technique. Fungal colonies were noted on PDA plates, and one greenish velvety colony was selected. Lactophenol Cotton Blue staining and microscopic evaluation of the colony revealed structures typical of Penicillium (brush-like conidiophores). Based on the colony observation and microscopic features, the isolate was identified as

Penicillium citrinum. This fungus has great industrial potential as it can utilize multiple carbon sources.

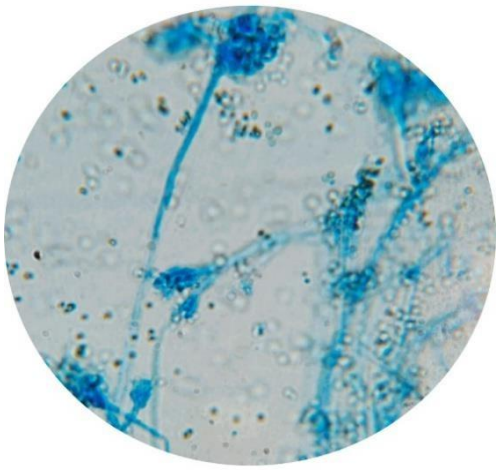


Fig.2 Microscopic morphology of *penicillium.sp* under (Higher magnification)

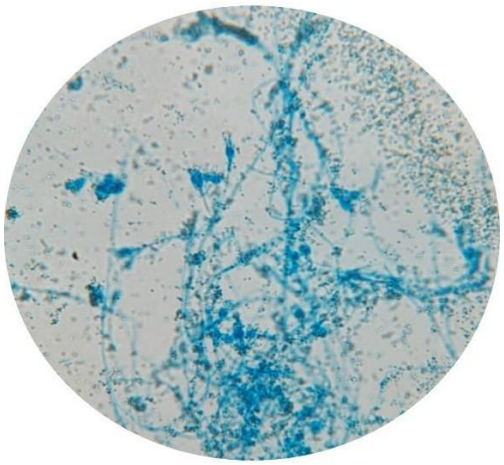


Fig.3 Microscopic morphology of *penicillium.sp* under (Lower magnification)

>PSF1

TGAACCTGCGGAAGGATCATTACCGAGTGCGGGCCCCTCGGGGCCCAACCTC
CCACCCGTGTTGCCCGAACCTATGTTGCCTCGGCGGGCCCCGCGCCCGCCGAC
GGCCCCCCTGAACGCTGTCTGAAGTTGCAGTCTGAGACCTATAACGAAATTAG
TTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGC
GAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTG
AACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCAT
TGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCCCCCGCCGGGGG
GACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGG
GGCTTCGTCACCCGCTCTAGTAGGCCCGGCCGGCGCCAGCCGACCCCCAACCT
TTAATTATCTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG
CATATCAATAAGCGAGAGGAA

Result: ***Penicillium citrinum***

Phylogenetic Tree

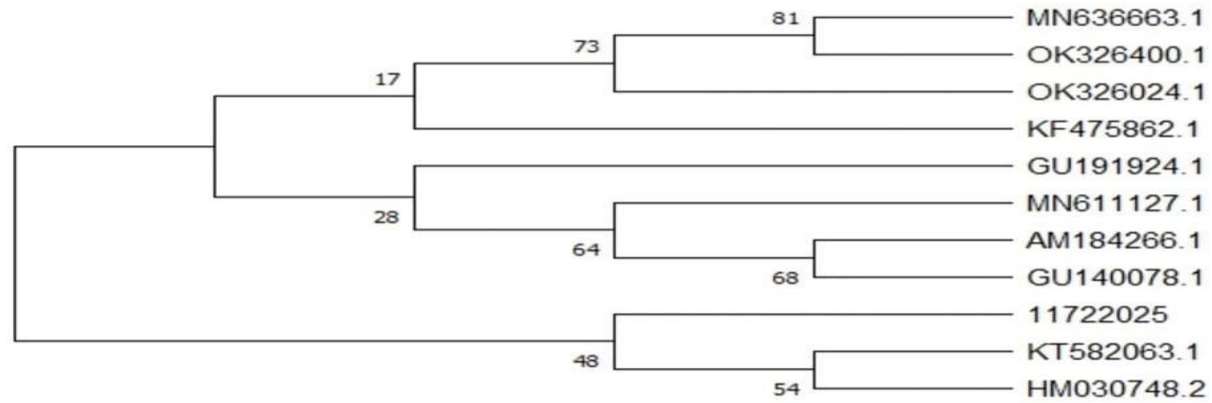


Fig.4.Molecular Identification and Phylogenetic Analysis of the Isolated Fungal Strain

Growth Observation during fermentation

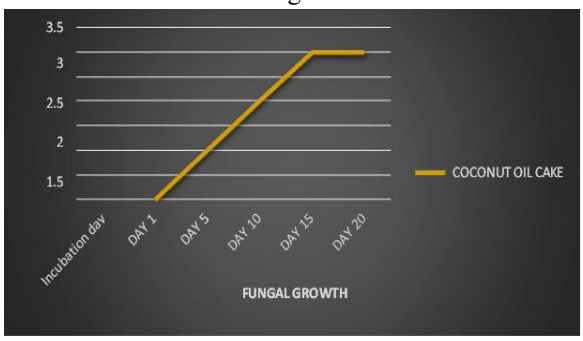


Fig.5 GROWTH KINETICS ON COCONUT OIL CAKE

Solid state fermentation was carried out with five agro-waste substrates. Fungal growth was found to be most prominent in coconut oil cake which was followed by rice bran and sugarcane bagasse. Growth of fungi was least in groundnut shell and groundnut oilcake. The differences in the level of production are likely due to differences in nutrient content and physical structure of the substrate. Penicillium fungi can grow on a wide range of substrates rich in organic material. The growth of fungi over time suggests that coconut oil cake is an acceptable substrate for solid-state fermentation. It continuously supported growth and biomass over time, which may indicate a useful substrate for bioprocesses that require degradation enzyme production, biodegradation, or secondary metabolite production and the inoculum size 10^{10}/ml spores is observed.

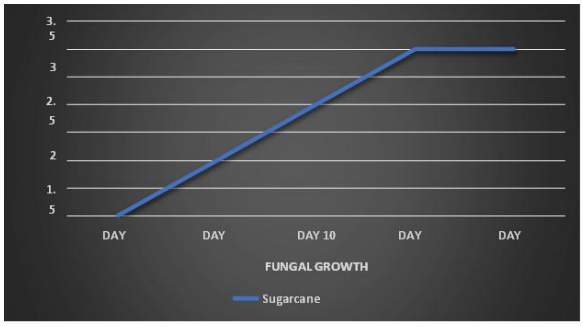


Fig.6 GROWTH KINETICS ON SUGARCANE BAGASSE

Sugarcane bagasse is known to naturally have high quantities of cellulose and hemicellulose; therefore, it will be widely used for fungi without major supplementation. What supports this fungal growth is the structural composition retains moisture and allows for adequate oxygen diffusion and formation of fungi biomass growth and development in solid-state fermentation. The results also suggest sugarcane bagasse is an ideal substrate for developing fungal bio-process processes like bio-enzymes, bio-pulping, or the bioconversion of agro-waste and the spores was observed as 10^8spore /ml in the sample.

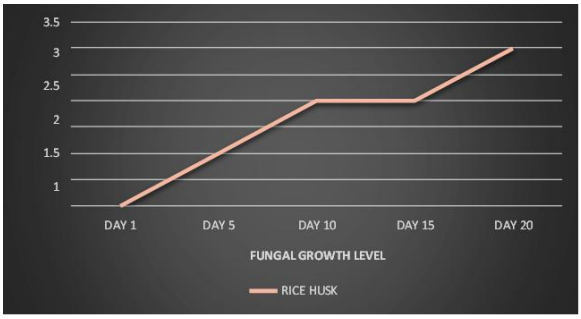


Fig.7 GROWTH KINETICS ON RICE HUSK

The high growth level of rice husk provides an ideal substrate, likely due to its porous nature and compositional characteristics from lignocellulosic ingredients and moisture retention which also indicates potential as a low-cost substrate for biotechnology applications like enzyme production, bioconversion, or myco remediation and the spores 10^5 spores /ml is noted.

Effect of PH

Fig.8 EFFECT OF PH LEVEL ON DIFFERENT SUBSTRATES

There was a slight fluctuation in the pH readings for the substrates. Coconut oil cake and rice husk had pH of about 6.0, which is condusive to growth of fungi and activity of enzymes leaving sugarcane bagasse with a lower pH (~5.0), thus more acidic.

Effect of Temperature

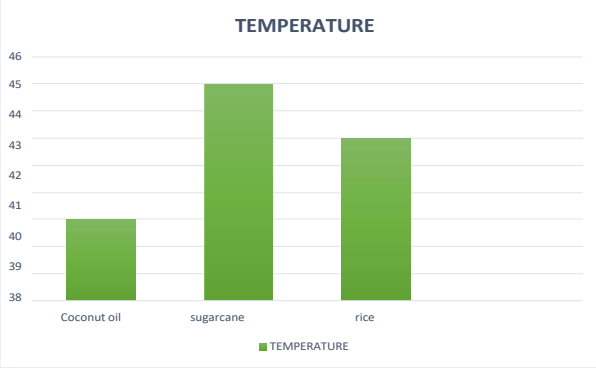


Fig.9 EFFECT OF TEMPERATURE ON DIFFERENT SUBSTRATES

Temperature profiles across the substrates had a moderate range. The maximum temperature was recorded in sugarcane bagasse (~45°C) and rice husk (~44°C) and the minimum temperature was closely similar with coconut oil cake (~40°C).

Effect of Moisture Content

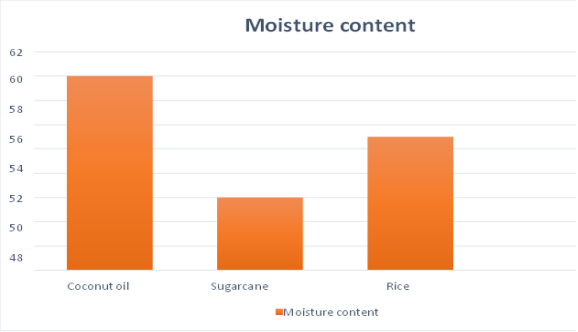


Fig.10 EFFECT OF MOISTURE LEVEL ON DIFFERENT SUBSTRATES

Moisture has a significant influence on solid state fermentation. Coconut oil cake had the highest moisture among the tested substrates (≈60%), followed by rice husk (≈55%), and sugarcane bagasse which had the lowest (≈50%).

Colorimetric Detection of Lovastatin

The crude ethanol extracts harvested following 20 days of fermentation were subjected to colorimetric detection, using a ferric chloride reagent. The product of lovastatin reacted with hydroxylamine through the lactone ring to form a reddish-brown complex in alkaline medium, which then formed a colored ferric-chelate from the complexation of ferric ions in acidic medium. Absorbance values were measured at 530 nm on a colorimeter. The maximum absorbance as recorded with coconut oil cake, followed by rice bran, and sugarcane bagasse, but minimal absorbance was recorded with the groundnut-based substrates.

FTIR analysis

All three substrates had the main functional groups associated with lovastatin (including the peaks for the ester carbonyl (C=O), methylene/methyl C-H stretching, and lactone C-O bands). However, coconut oil cake had the sharpest and most defined peaks (especially for the ester carbonyl and lactone peaks), which may relate to a higher concentration of lovastatin in coconut oil cake. This higher concentration may reflect the fact that coconut oil cake had more lipids and nutrients which may have boosted secondary metabolism in the fungus. Sugarcane bagasse had similar strong signals for lovastatin indicating that it also worked effectively as a fermentation substrate. Rice husk had peaks for lovastatin confirming its presence; however, its peaks were relatively more broad or less intense which may relate to the higher silica of rice husk interfering with the extraction or FTIR FTIR analysis confirmed that lovastatin was present in the extracts of all three substrate-based systems, so our fermentation process was effective. Based on the results, coconut oil cake appears to be the best substrate for the production of lovastatin under the tested conditions.

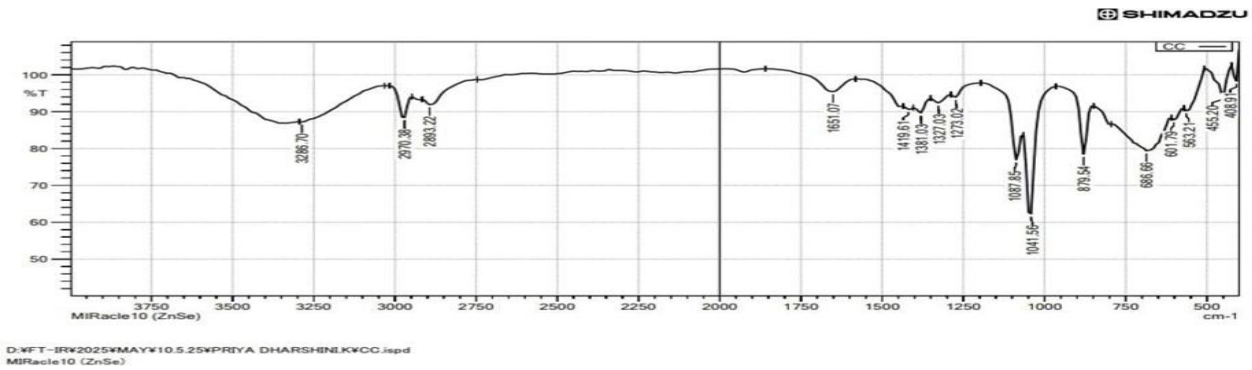


Fig.11 FTIR SPECTRAL ANALYSIS OF COCONUT OIL CAKE

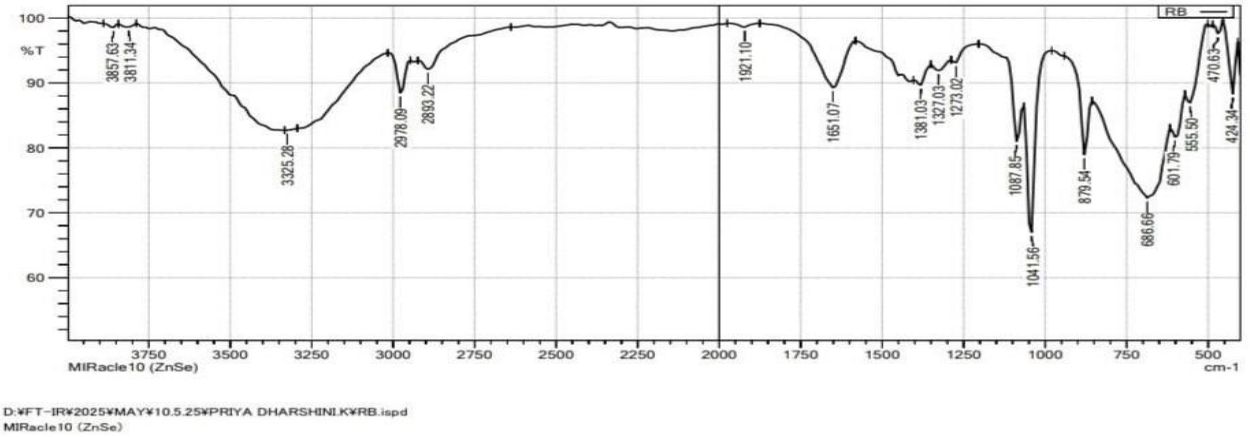


Fig.12 FTIR SPECTRAL ANALYSIS OF RICE HUSK

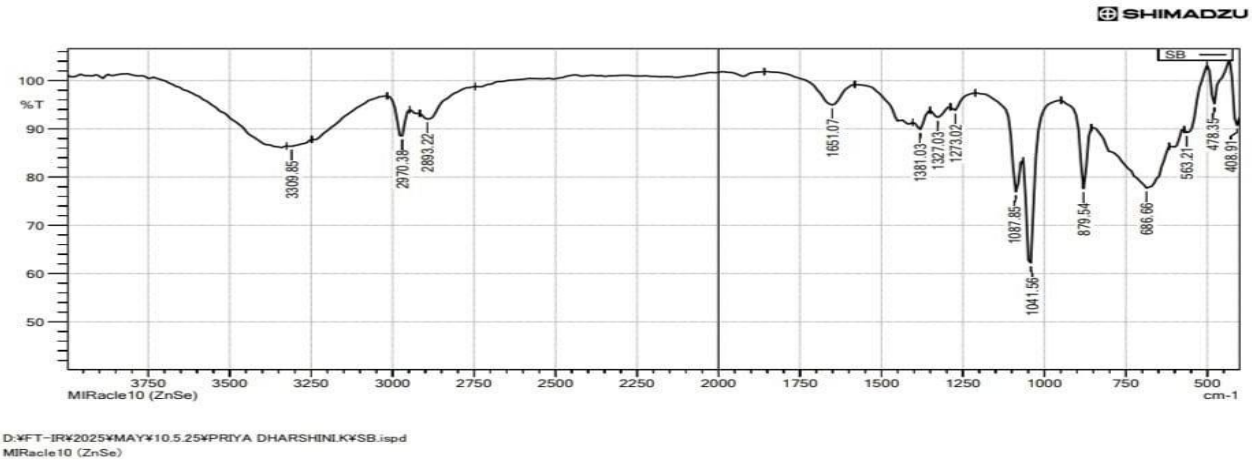


Fig.13 FTIR SPECTRAL ANALYSIS OF SUGARCANE BAGASSE

DISCUSSION

The morphological properties observed in this study, such as bluish velvety colonies on PDA and brush-like conidiophores under lactophenol cotton blue staining, were comparable to the characterizations for *Penicillium citrinum* outlined by [13]. The molecular identification of the isolates also further verified the morphological characteristics, as the sequence alignment yielded a high similarity to known *P. citrinum* strains deposited in GenBank. *Penicillium citrinum* is widely dispersed in soil environments and recognized for its production of several secondary metabolites, including citrinin and lovastatin [25]. The organism's ability to utilize several carbon sources highlights its promise as a valuable industrially and pharmaceutical used fungal strain. The phylogenetic analysis shows that the isolate is closely clustered with several other *P. citrinum* strains from various geographical locations. This indicates that *P. citrinum* shows little genetic variability and that it displays great adaptability geographically. Overall, it was successful to isolate *P. citrinum* from Gudalur soil, and highlights the significance of local soil habitats to yield valued fungal strains that can be used industrially. Furthermore, studies that focus on the metabolite profile and induce and optimize fermentation conditions can develop its entire biotechnological value with larger emphasis to bioactive compounds such as lovastatin.

The fungal growth on coconut oil cake was observed every five days for 20-day incubation shown in Fig. 5. Day 1: No visible growth was observed in the sample indicating the organism was in the lag phase/adaptation period (Growth Level: 0). Day 5: Light growth was observed on the sample, indicating the beginning of fungal growth and development, as

part of the fungal organism began adapting to the substrate environment. Day 10: Moderate growth levels were observed, indicating the individual was undergoing increased metabolic activity and nutrient assimilation and the is observed as 10^4spores /ml spores in the sample. Day 15 & Day 20: High/maximum growth was observed from Day 15 through to Day 20, indicating the total colonisation and saturation of the substrate, and the inoculum size value was observed as 10^8spores/ml in the sample. The growth pattern is one of gradual increase, reaching a plateau at a high level of growth after Day 15. This growth pattern indicates that coconut oil cake provides a moderately rich substrate with sufficient nutrients to sustain fungal growth. The oil and protein content may provide additional energy and growth after the lag phase. However, the plateau at Day 15 indicates a potential lack of nutrients or limited space, which indicates that it is the stationary phase of the fungal growth and the inoculum size 10^{10}/ml spores is observed.

The fungal growth kinetics on the sugarcane bagasse substrate Fig.6 with growth data collected every 5-days for 20-days of incubation. Day 1: No growth was observed (lag phase). Day 5: Slight growth (the first observed growth stage) the growth was maintained and 10^2 spores/ml is observed. Day 10: Moderate growth, continued growth activity, and nutrient uptake and observed spore is 10^4 spores/ml. Day 15 & Day 20: High or maximum growth was maintained and 10^8spore/ ml was observed. The trends show a consistent linear and continuous growth curve, which is expected because sugarcane bagasse is an excellent substrate with effective porosity and lignocellulosic composition. And growth reached the stationary phase on Day 15, as seen in other substrates and the spores was observed as 10^8spore /ml in the sample.

The growth kinetics of fungi on rice husk substrate Fig.7 were monitored over the 20-day incubation period. Day 1: No visible Fungal growth was present on the rice husk (Growth Level: 0). This indicated that it undergoes a lag phase. Day 5: Some initial growth of fungi was evident, indicating the process of colonization had started, as the fungal spores began to use the available nutrients (Growth Level: 1) and the inoculum size is 10^2spore /ml is noted. Day 10: Moderate fungal growth was seen, indicating adaptations had occurred and that the fungal species' metabolism started to increase and the inoculum size is 10^3spore /ml. Day 15 & Day 20: The maximum and/or extensive level of fungal growth was observed. The growth did appear to plateau around Day 15, and the spores observed is 10^4 spores/ml. which may suggest that the substrate reached Saturation points or the fungi had used the available nutrients as much as possible (Growth Level: 3 on Day 15 & Day 20. These results show a typical sigmoidal growth pattern with a lag phase (Day 1), log phase (Day 5-Day 10), and stationary phase (Day 15) that continued through Day 20. The rice husk supported persistent fungal growth, revealing it has potential as a substrate for fungal cultivation or solid-state fermentation.

The degree of pH variance may affect the microbial activity and competing growth patterns during fermentation. The differences in temperature could be attributed to the metabolic heat from fungal activity and the thermal conductivity of the substrates. These temperatures were in a good range to ensure that fungal fermentation would occur for good biomass degradation. An increase in moisture content usually favors fungal growth, but too much moisture can also lead to anaerobic conditions. Therefore, the moisture content of coconut cake could underlie the rapid and vigorous fungal growth seen in the previous growth kinetics [37].

The maximum absorbance as recorded with coconut oil cake, followed by rice bran, and sugarcane bagasse, but minimal absorbance was recorded with the groundnut-based substrates. The colorimetric method used in this study followed the procedure outlined by [36], as a validated and reliable method to estimate the incomparable quantity of lovastatin concentrations from biological samples and extracts.

To confirm the presence of lovastatin in the fermented products based on coconut oil cake, sugarcane bagasse, and rice husk substrates the FTIR spectral analysis is outlined below. Lovastatin is a polyketide with characteristic functional group peaks in the FTIR, such as: Sharp ester C=O stretching peak around 1735 - 1745 cm^{-1}, Methyl and methylene C–H stretching bands at 2920 - 2850 cm^{-1}, Lactone ring (cyclic ester) vibrations at wavelengths in the 1100 - 1300 cm^{-1} region, Broad O–H stretching near 3400 cm^{-1}

attributed to hydroxyl groups.[12].

The FTIR spectrum of the extract of the coconut oil cake substrate had a sharp absorption peak at around 1745 cm^{-1} which can be attributed to the ester carbonyl group, which is indicative of the lactone ring of the lovastatin molecule. Additional peaks at 2923 cm^{-1} and 2853 cm^{-1} correspond to aliphatic C–H stretching, and the broad peak around 3440 cm^{-1} relates to hydroxyl groups. The peaks that appear in the C–O stretching bands located close to 1160 cm^{-1} further support the lactone structure located in the molecule. These peaks are consistent with the known FTIR profile of lovastatin, thus indicating that lovastatin is present in the sample. In the FTIR spectrum of the extract from sugarcane bagasse, there was a very clear C=O peak for an ester at about 1735 cm^{-1}, C–H stretching bands were seen at 2924 cm^{-1} and 2854 cm^{-1}, and a broad peak was observed at 3430 cm^{-1} for O–H. In addition, bands in the region of 1160– 1120 cm^{-1} corresponded to C–O stretching, allowing us to suggest that a lactone ring is present. The data overall provided clear support for the presence of lovastatin in the extract from this substrate. The FTIR profile for the extract from rice husk also demonstrated C=O stretching vibration at about 1736 cm^{-1}, C–H bands were observed in the same 2920-2850 cm^{-1} region, and a broad hydroxyl peak was seen at 3415 cm^{-1}. Also, C–O vibrational peaks in the region of about 1125 cm^{-1}, which correspond well to known functional groups for lovastatin were observed. All of the peaks showed good evidence for the successful biosynthesis of lovastatin using sources based on rice husk.

CONCLUSION

This study was developed to produce lovastatin, a secondary metabolite with cholesterol lowering ability, using *Penicillium citrinum* isolated from soil utilizing solid-state fermentation. The study considered using agro-industrial wastes (coconut oil cake, sugarcane bagasse, rice bran, groundnut shell and groundnut oil cake) as substrates for fungal growth and metabolite yield. The fungus was characterized and identified by culture morphology and microscopy, and grown under laboratory conditions. All substrates contain lactose monohydrate as carbon source and ammonium sulfate and yeast extract as nitrogen sources. The fungi were fermented for 20 days at 28 ± 2 °C static conditions. After fermentation recovered biomass was extracted with ethanol and then analyzed for lovastatin by colorimetric method and FTIR analysis.

The lovastatin was confirmed calorimetrically by comparing absorbance at 530 nm with a red- brown complex formed. Coconut oil cake had the most intense color compared to all substrates indicating the highest lovastatin level, followed by rice bran and sugarcane bagasse. There was low production for groundnut oil cake and groundnut shell. FTIR analysis was also successful in confirming the existence of the functional groups associated with lovastatin, including ester carbonyl, hydroxyl and lactone functional groups. These spectral results validated the results of the colorimetric test. In summary, it was shown that *Penicillium citrinum* has effective seizure of lovastatin production in solid state fermentation. Of the agricultural waste substrates, coconut oil cake was identified as the best suitable agro-waste substrate for yielding lovastatin. The current work demonstrated the potential to produce pharmaceutically valuable compounds cost-effectively and in a sustainable manner using native fungal strains and low-value agricultural residues.

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