Production of Lovastatin using Liquid Cheese Whey by *Fusarium nectrioides* (MH173849), an Endophytic Fungi Isolated from *Euphorbia hirta*

Senthamarai Manogaran1, Kannan Kilavan Packiam1, Vijayakumar Lakshmi Narayanan2, Chadhurthika Krishnamurthy1, Devi Vijayarangam1 and Moni Philip Jacob Kizhakedathil1

1Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode, Tamil Nadu India.
2Department of Pharmaceutical Technology, Hindusthan Institute of Technology, Valley Campus, Coimbatore, Tamil Nadu, India.

Abstract

Lovastatin is a naturally produced 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzyme inhibitor- used for treating hypercholesterolemia. It was the first statin drug which was approved by the United States Food and Drug Administration (USFDA). In the current study, endophytic fungus *Fusarium nectrioides* (MH173849) isolated from *Euphorbia hirta* L. was used for the production of lovastatin. Four different culture media indicated as M1, M2, M3 and M4 were used for the initial production of lovastatin. Liquid cheese whey was used as nitrogen source. Growth morphology of fungi was investigated using Scanning Electron Microscopy analysis. Also, parameters like temperature, pH, inoculum size, incubation time, and RPM were optimized for the obtaining highest lovastatin production. Among the four media, M4 was found to produce the maximum concentration of lovastatin. Parameters such as temperature of 28°C, pH 6, RPM – 180 rpm and inoculum size of 5 x10⁷ spores/mL were optimal for the production of lovastatin by *F. nectrioides* (MH173849).

Keywords: *Fusarium nectrioides*, Lovastatin, Endophytic Fungi, *Euphorbia hirta* (Linn), Liquid Cheese Whey, Optimization
INTRODUCTION

Diseases like hypercholesterolemia, i.e., increased blood cholesterol levels, have been on the rise due to changes in diet and lifestyle across the globe. Elevated cholesterol levels in the blood pose a significant threat as it increases the likelihood of a person developing atherosclerosis and ischemic heart disease in the long run. Research indicated that it could also increase the chances of getting diseases such as dementia, Alzheimer’s, Ischemic heart stroke, obesity, diabetes, and certain cancers. The liver predominantly synthesizes cholesterol in a series of more than 25 enzyme-driven steps, and the rest is derived from the food we consume\textsuperscript{1,2}. Biosynthesis of cholesterol in the body takes place through the mevalonic acid pathway. The predominant enzyme targeted for controlling the cholesterol level is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting and regulatory enzyme in cholesterol biosynthesis. The group of compounds that inhibit the enzyme HMG-CoA reductase is the statin group of molecules\textsuperscript{1,3}.

Lovastatin is a statin group of a drug molecule and was the first anti-cholesterol drug approved by the United States Food and Drug Administration (USFDA). It is involved in the inhibition of cholesterol biosynthesis. Lovastatin also works as an in vivo competitive inhibitor of HMG-CoA reductase for humans and animals as well as in plants and is hence used to treat hypercholesterolemia\textsuperscript{1,3,8}. Lovastatin is available in the lactone form but converted to β-hydroxy acid in vivo, the active state. Also, Lovastatin is the precursor of simvastatin\textsuperscript{4}. Studies have shown that lovastatin can also increase good cholesterol or high-density lipoproteins in the blood, thereby preventing plaque formation\textsuperscript{6}. Apart from its anti-cholesterol activity, lovastatin has anti-inflammatory, anti-cancer, neuro-protective, and antimicrobial properties. Studies have indicated that it could be effective against osteoporosis, Alzheimer’s disease, and multiple sclerosis\textsuperscript{1,7}.

Literature has shown that fungal fermentation has yielded several biologically important active metabolites. Several fungal species can produce lovastatin by solid-state, liquid surface, and submerged fermentation. Lovastatin is produced intracellularly and accumulates in the mycelia. Fungal species such as Aspergillus terreus, Aspergillusflavus, Penicillium spp., Monascusruber, Monascuspurpureus, Trichoderma spp. are commonly employed for the production of lovastatin. Also, Scopulariopsis, Paecilomyces, Phoma, Gymnoascus, Doratomyces, Pleurotus, Phytophthora, and Hypomyces have shown the potential to produce lovastatin. Mushrooms like Agaricus bisporus, Pleurotusostreatus, and P. citrinopileatus have been used to produce lovastatin\textsuperscript{1,2,4,8-10}.

Endophytic fungi exist in the tissues of living plants and aid in the growth of plants. These fungi protect the plant from biotic and abiotic stresses and don’t cause any harm to the plants. In turn, the plant provides these fungi with nutrition and shelter. Endophytic fungi produce secondary metabolites and bioactive compounds that are structurally unique with diverse pharmacological properties. So, research has been focused on producing such bioactive products from endophytes so that they may be used in the treatment of diseases\textsuperscript{11-18}. In the present study, we did a preliminary investigation on the potential of the endophytic fungus Fusariumnectrioides for the production of lovastatin.

MATERIALS AND METHODS

Isolation of \textit{Fusariumnectrioides}(MH173849) from \textit{E. hirta}

\textit{Fusariumnectrioides}(MH173849), an endophytic fungus was isolated from the medicinal plant, \textit{Euphorbia hirta}. \textit{F. nectrioides} were investigated for lovastatin production. The isolated pure cultures were maintained in a potato dextrose agar culture medium (PDA). It is involved in the inhibition of cholesterol biosynthesis. Lovastatin also works as an in vivo competitive inhibitor of HMG-CoA reductase for humans and animals as well as in plants and is hence used to treat hypercholesterolemia\textsuperscript{1,3,8}. Lovastatin is available in the lactone form but converted to β-hydroxy acid in vivo, the active state. Also, Lovastatin is the precursor of simvastatin\textsuperscript{4}. Studies have shown that lovastatin can also increase good cholesterol or high-density lipoproteins in the blood, thereby preventing plaque formation\textsuperscript{6}. Apart from its anti-cholesterol activity, lovastatin has anti-inflammatory, anti-cancer, neuro-protective, and antimicrobial properties. Studies have indicated that it could be effective against osteoporosis, Alzheimer’s disease, and multiple sclerosis\textsuperscript{1,7}.

Literature has shown that fungal fermentation has yielded several biologically important active metabolites. Several fungal species can produce lovastatin by solid-state, liquid surface, and submerged fermentation. Lovastatin is produced intracellularly and accumulates in the mycelia. Fungal species such as Aspergillus terreus, Aspergillusflavus, Penicillium spp., Monascusruber, Monascuspurpureus, Trichoderma spp. are commonly employed for the production of lovastatin. Also, Scopulariopsis, Paecilomyces, Phoma, Gymnoascus, Doratomyces, Pleurotus, Phytophthora, and Hypomyces have shown the potential to produce lovastatin. Mushrooms like Agaricus bisporus, Pleurotusostreatus, and P. citrinopileatus have been used to produce lovastatin\textsuperscript{1,2,4,8-10}.

Endophytic fungi exist in the tissues of living plants and aid in the growth of plants. These fungi protect the plant from biotic and abiotic stresses and don’t cause any harm to the plants. In turn, the plant provides these fungi with nutrition and shelter. Endophytic fungi produce secondary metabolites and bioactive compounds that are structurally unique with diverse pharmacological properties. So, research has been focused on producing such bioactive products from endophytes so that they may be used in the treatment of diseases\textsuperscript{11-18}. In the present study, we did a preliminary investigation on the potential of the endophytic fungus \textit{Fusariumnectrioides} for the production of lovastatin.

MATERIALS AND METHODS

Isolation of \textit{Fusariumnectrioides}(MH173849) from \textit{E. hirta}

\textit{Fusariumnectrioides}(MH173849), an endophytic fungus was isolated from the medicinal plant, \textit{Euphorbia hirta}. \textit{F. nectrioides} were investigated for lovastatin production. The isolated pure cultures were maintained in a potato dextrose agar culture medium (PDA). It is involved in the inhibition of cholesterol biosynthesis. Lovastatin also works as an in vivo competitive inhibitor of HMG-CoA reductase for humans and animals as well as in plants and is hence used to treat hypercholesterolemia\textsuperscript{1,3,8}. Lovastatin is available in the lactone form but converted to β-hydroxy acid in vivo, the active state. Also, Lovastatin is the precursor of simvastatin\textsuperscript{4}. Studies have shown that lovastatin can also increase good cholesterol or high-density lipoproteins in the blood, thereby preventing plaque formation\textsuperscript{6}. Apart from its anti-cholesterol activity, lovastatin has anti-inflammatory, anti-cancer, neuro-protective, and antimicrobial properties. Studies have indicated that it could be effective against osteoporosis, Alzheimer’s disease, and multiple sclerosis\textsuperscript{1,7}.

Literature has shown that fungal fermentation has yielded several biologically important active metabolites. Several fungal species can produce lovastatin by solid-state, liquid surface, and submerged fermentation. Lovastatin is produced intracellularly and accumulates in the mycelia. Fungal species such as Aspergillus terreus, Aspergillusflavus, Penicillium spp., Monascusruber, Monascuspurpureus, Trichoderma spp. are commonly employed for the production of lovastatin. Also, Scopulariopsis, Paecilomyces, Phoma, Gymnoascus, Doratomyces, Pleurotus, Phytophthora, and Hypomyces have shown the potential to produce lovastatin. Mushrooms like Agaricus bisporus, Pleurotusostreatus, and P. citrinopileatus have been used to produce lovastatin\textsuperscript{1,2,4,8-10}.

Endophytic fungi exist in the tissues of living plants and aid in the growth of plants. These fungi protect the plant from biotic and abiotic stresses and don’t cause any harm to the plants. In turn, the plant provides these fungi with nutrition and shelter. Endophytic fungi produce secondary metabolites and bioactive compounds that are structurally unique with diverse pharmacological properties. So, research has been focused on producing such bioactive products from endophytes so that they may be used in the treatment of diseases\textsuperscript{11-18}. In the present study, we did a preliminary investigation on the potential of the endophytic fungus \textit{Fusariumnectrioides} for the production of lovastatin.

MATERIALS AND METHODS

Isolation of \textit{Fusariumnectrioides}(MH173849) from \textit{E. hirta}

\textit{Fusariumnectrioides}(MH173849), an endophytic fungus was isolated from the medicinal plant, \textit{Euphorbia hirta}. \textit{F. nectrioides} were investigated for lovastatin production. The isolated pure cultures were maintained in a potato dextrose agar culture medium (PDA). It is involved in the inhibition of cholesterol biosynthesis. Lovastatin also works as an in vivo competitive inhibitor of HMG-CoA reductase for humans and animals as well as in plants and is hence used to treat hypercholesterolemia\textsuperscript{1,3,8}. Lovastatin is available in the lactone form but converted to β-hydroxy acid in vivo, the active state. Also, Lovastatin is the precursor of simvastatin\textsuperscript{4}. Studies have shown that lovastatin can also increase good cholesterol or high-density lipoproteins in the blood, thereby preventing plaque formation\textsuperscript{6}. Apart from its anti-cholesterol activity, lovastatin has anti-inflammatory, anti-cancer, neuro-protective, and antimicrobial properties. Studies have indicated that it could be effective against osteoporosis, Alzheimer’s disease, and multiple sclerosis\textsuperscript{1,7}.

Literature has shown that fungal fermentation has yielded several biologically important active metabolites. Several fungal species can produce lovastatin by solid-state, liquid surface, and submerged fermentation. Lovastatin is produced intracellularly and accumulates in the mycelia. Fungal species such as Aspergillus terreus, Aspergillusflavus, Penicillium spp., Monascusruber, Monascuspurpureus, Trichoderma spp. are commonly employed for the production of lovastatin. Also, Scopulariopsis, Paecilomyces, Phoma, Gymnoascus, Doratomyces, Pleurotus, Phytophthora, and Hypomyces have shown the potential to produce lovastatin. Mushrooms like Agaricus bisporus, Pleurotusostreatus, and P. citrinopileatus have been used to produce lovastatin\textsuperscript{1,2,4,8-10}.

Endophytic fungi exist in the tissues of living plants and aid in the growth of plants. These fungi protect the plant from biotic and abiotic stresses and don’t cause any harm to the plants. In turn, the plant provides these fungi with nutrition and shelter. Endophytic fungi produce secondary metabolites and bioactive compounds that are structurally unique with diverse pharmacological properties. So, research has been focused on producing such bioactive products from endophytes so that they may be used in the treatment of diseases\textsuperscript{11-18}. In the present study, we did a preliminary investigation on the potential of the endophytic fungus \textit{Fusariumnectrioides} for the production of lovastatin.
Production Medium
In order to study the production of lovastatin, *F. nectrioides* was inoculated in four different media. The four media are Potato Dextrose Broth (M1), Potato Dextrose Broth with Liquid Cheese Whey (M2), Modified media (M3) and modified media with Liquid Cheese Whey (M4). Whey is a liquid by-product generated from cheese manufacturing. It is composed of approximately 0.3% fat, 0.8% protein, 4.9% lactose, and 0.5% minerals. Liquid Cheese Whey is used as both a carbon and nitrogen source. Spore inoculum in seed media (5x10^7 spore per mL) was inoculated in the M1-M4 production media in triplicates under sterile conditions. The pH of the media was maintained at pH 6. All the flasks were incubated at 28°C at 180 rpm for 15 days.

The composition of the four media used are as follows,
- **M1 media** - Potato Dextrose Broth - 250 g of peeled, sliced potatoes were boiled in 1 L distilled water for 30 min and filtered afterwards. With the 1 L of filtrate, 20g of dextrose was added.
- **M2 media** - Potato Dextrose Broth with Liquid Cheese Whey - 20g of acidified Liquid Cheese Whey was added to M1 medium.
- **M3 medium** - Modified Media - contained: glucose (20g/L), lactose (20g/L), yeast extract (20g/L), histidine (4g/L), potassium di hydrogen phosphate (4g/L), Magnesium Sulphate (0.4g/L), Calcium chloride (0.4g/L), Ferrous sulphate heptahydrate (0.2g/L) was dissolved in 1000ml distilled water.
- **M4 medium** - Modified Media with Liquid Cheese Whey - Lactose from M3 medium was re-placed with Liquid Cheese whey.

**Extraction and Detection of Lovastatin**
Fermentation of the *F. nectrioides* in the four different media was carried out for about 16 days. The media was filtered using sterile filter paper to separate the mycelia from the media. Then, equal volumes of ethyl acetate were added to the filtered media, and the solution was acidified from pH 6 to pH 2. The solution was kept in a shaker at 100 rpm for 2h at room temperature. After the extraction cycle, the mixture was centrifuged at 1500 rpm for 20 min, and the organic phase was collected. It was then concentrated using a rotary evaporator. The extract was then analysed for lovastatin production using UV spectrometry at 238nm.

**SEM Analysis of Fungal Growth on Four Media**
The various growth morphology of *F. nectrioides* in the four different culture media M1, M2, M3, and M4 were investigated using a Scanning Electron Microscope (SEM). Biomass separated from the culture broth by centrifugation was washed with sterile distilled water and stored in alyophilizer at 20°C. Lyophilized samples were dissolved in dimethyl sulfoxide (DMSO) and used for analysis.

**Effect of Culture Conditions (Temperature, pH, Inoculum Size, Incubation Time, and RPM) on Fungal Growth and Lovastatin Production**
Based on preliminary analysis, Modified media with liquid cheese whey (M4) gave a high yield of lovastatin. This was then chosen for further optimization studies. Effect of different temperatures, pH, inoculum size, incubation time, and RPM in the shaker flask level fermentation was studied to enhance the Lovastatin production.

**RESULTS**

**Production, Extraction, and Confirmation of Lovastatin**
*Fusarium nectrioides* isolated from *Euphorbia hirta* were investigated for the production of Lovastatin. The pure cultures maintained in PDA were initially inoculated in the seed medium. The *F. nectrioides* were inoculated in four different media from the seed media, i.e., M1–M4. The cultures were allowed to grow in the production media for about 16 days, and the production was monitored till the 16th day of fermentation. After which, ethyl acetate was added to the fermentation broth to extract lovastatin. After the extraction, the presence of lovastatin was confirmed in all four media by UV spectrophotometry at 238nm. The quantitative estimation was done by comparing the values with the lovastatin standard curve.
M1 and M3 media, the maximum concentration of lovastatin produced was 12.7 g/L and 14.3 g/L, respectively, on the 10th day of incubation. In M2 media, maximum lovastatin production was observed on the 11th day when titers reached 11.3 g/L lovastatin. Among the four media, the maximum production was observed in M4 media, which yielded 16.4 g/L lovastatin on the 9th day of incubation. So lovastatin production was focused on M4 media containing liquid cheese whey for further optimization studies (Figure 1).

**SEM Analysis of Fungal Growth on Four Media**

The morphological growth pattern of *F. nectrioides* in all four culture media was analyzed under the Scanning electron microscope and is represented in Figure 2. Compared to other media, M4 media showed dense growth of fungus and higher biomass which designates that the components of modified media, in combination with liquid cheese whey supported the fungus in terms of their growth and product formation.

**Effect of Culture Conditions on Fungal Growth and Lovastatin Production**

Growth of fungus and lovastatin production was observed in the M4 culture medium. Specific parameters of M4 culture media such as temperature, pH, RPM, inoculum size, and the incubation period were monitored. Based on the optimization studies, maximum lovastatin production was observed at a temperature of 28°C, pH of 6, RPM of 180, and inoculum size of 5 x10^7 spores/mL on the 9th day.

For optimizing the temperature, the temperature of M4 media varied between 22°C and 30°C. The maximum production of lovastatin was observed at 28°C on the 9th day. Literature suggests that when the temperature in the production media was maintained outside the optimum temperature range, it impacted the metabolic activities of the microorganisms. At temperatures lower or higher than the optimum temperature, the yield of the lovastatin was low. The decreased production of lovastatin at high temperatures could be attributed to the

![Figure 1](image-url). Effect of four media – M1, M2, M3 and M4 media on the production of lovastatin.
inactivation or the denaturation of lovastatin, but not only. Also, when the incubation period is prolonged, lovastatin production decreases. This might be possibly due to the cultures reaching the end-point of fermentation. These results coincide with previously published data on lovastatin production by Monascusruber, Monascuspurpureus, and Aspergillus terreus (Figure 3).

For optimizing the pH, the pH of M4 media ranged between 4 and 8. The maximum production of lovastatin was observed at pH=6 on the 11th day. pH plays an important role in sustaining all biochemical processes and therefore, it can affect the growth of fungi and its capability to produce metabolites. pH has a major influence on the production of lovastatin. Literature suggests that pH between 5.8 and 7.5 are optimal for the production of lovastatin whereas the acidic pH of the media inhibits lovastatin production. The pH that was used in the current study i.e., pH 6 aids in the production of lovastatin (Figure 4). The rpm of M4 media was varied between 120 and 200 rpm to optimize the rpm. The maximum production of lovastatin was observed at 180 on the 11th day. It was observed that the rpm less than 180 provided insufficient oxygen to the fungal cells. When the rpm is increased, the cells might have experienced shear stress thereby there, a decrease in the production of lovastatin was observed (Figure 5).

The inoculum concentration plays an essential role in lovastatin production. The maximum lovastatin production was observed at 5 x 10^7 spores/mL on the 9th day. As the inoculum size increased, a decrease in lovastatin production was observed. An increase in the inoculum size may have shifted the dynamics towards growth rather than lovastatin production. It was observed that when the inoculum concentration was more, the lovastatin production was around
the 7th day. But the yield of lovastatin was low (Figure 6). To estimate the optimum incubation period, fermentation flasks were incubated for 16 days. The production of lovastatin was observed from day 2 of incubation. A steady increase in lovastatin production was observed up to day 9;

Figure 3. Effect of temperature on the production of lovastatin in M4 media.

Figure 4. Effect of pH on the production of lovastatin in M4 media.
after that, a decline in lovastatin production was seen. They indicated that 9 days of incubation was sufficient for the maximum lovastatin production. Previous reports indicated that incubation between 6 and 10 days was optimum for lovastatin production by different fungi (Figure 7).

**DISCUSSION**

For enhancing the production of lovastatin, four different culture media have been formulated by replacing the lactose with a Liquid cheese Whey as one of the carbon sources, and the morphological growth variations of *F. nectrioides* were investigated using SEM analysis. In addition to the morphological variations, the culture conditions of the chosen fungi in medium M4 were found to be best at a temperature of 28°C, pH of 6, and RPM of 180 for the growth of *F. nectrioides* (MH173849). Due to its wide applications in medicinal fields, demand for this compound increased many folds and required a strong science base, cheap raw materials, and effective fungal isolates to meet the requirement for commercial production. So, the novel idea of utilizing industrial waste, such as Liquid Cheese Whey has emerged for the cost-effective production of lovastatin at a large scale using *F. nectrioides*.

Lovastatin is a medication given to patients to manage high cholesterol levels. It exists in two forms. The active form of lovastatin is an open-ring β-hydroxy acid and the inactive form is the closed-ring β-lactone form. The properties of these two forms i.e. pharmaceutical and physicochemical properties are different. In general, the filamentous fungi secretes the lovastatin in the active open-ring β-hydroxy acid form in the culture media. But the lactone form of lovastatin is beneficial for quantification analyses. So, the β-hydroxy acid form needs to be converted to lactone form. This is achieved by reducing the pH and lactonization form. Therefore, producing lovastatin in an acidic medium is required.

The physical and chemical parameters that are maintained in growth media conditions have an effect on the production of secondary metabolites by the fungi. In general, the synthesis of secondary metabolites occurs in the late log phase or the stationary phase of growth. This is owing to the fact that the essential nutrients get depleted in the late log phase. It cannot secrete or synthesize at the early growth stage of fungi. Also, the secretion...
**Figure 6.** Effect of spore concentration on the production of lovastatin in M4 media.

**Figure 7.** Effect of number of days for the Lovastatin production in M4 media.
of accumulated metabolites into the surrounding medium is necessary. Lovastatin production is highly influenced by slow metabolizing carbon sources like lactose, glycerol, and fructose when compared to glucose34-36. Fungi produce lovastatin as a secondary metabolite. Lovastatin production in fungi uses the pathway which utilizes slow metabolizing carbon. Whereas, it uses carbon sources like glucose biomass production. Thus, lactose used in the medium favors the production of lovastatin37.

In the literature, many have reported on the production of lovastatin. Jaivel and Marimuthu, reported the production of lovastatin by A. terreus (JPM3)37. They have reported that A. terreus (JPM3), yielded 138.4 mg L\(^{-1}\)lovastatin. In another study, Pecyna and Bizukojcanalyzed lovastatin yield in submerged fermentation using the lactose-to-glycerol ratio. The yield of lovastatin was about161.8 mg L\(^{-1}\)38. Sridevi and Charya, reported the isolation and production of lovastatin by A. terreus KSVL-SUCP-7539. The maximum production of lovastatin of 360 mg L\(^{-1}\) was observed. A. terreus KPR12 produced a maximum lovastatin yield of 450.79 mg L\(^{-1}\) which was reported by Srinivasan et al.33. Mahmoud and Hadi reported Laetioporus sulphurous which was reported to produce maximum lovastatin of about 280μg/mL40. Pushpa et al. reported lovastatin production from various fungi such as Shizophyllum commune and Pleurotusostreatus which produced 38μg/mL and 30μg/mL, respectively33.

CONCLUSION
From this study, culture medium M4 was found to work best at a temperature of 28°C, pH of 6 and RPM of 180 for the growth of F. nectrioides (MH173849). Hence, industrial waste i.e., liquid cheese whey could be used as one of the carbon and nitrogen source for the production of Lovastatin. Further studies need to be performed to further scale-up the production.

ACKNOWLEDGMENTS
The authors are thankful to the Management of Bannari Amman Institute of Technology, Sathyamangalam for providing the necessary facilities.

CONFlict OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING
None.

DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
Not applicable.

REFERENCES
1. Easa SM, Mattar ZA, Khalaf MA, Khalil MF. Biosynthesis of Lovastatin by Gamma Irradiated Aspergillus terreus. J Nucl Sci Technol. 2021; 9(1):19-31. doi: 10.21608/jntas.2021.54136.1031
2. Hakiem AF, Mohamed NA, Ali HR. FTIR spectroscopic study of two isostructural statins: Simvastatin and Lovastatin as authentic and in pharmaceuticals. Spectrochim Acta A Mol Biomol Spectrosc. 2021; 261:120045. doi: 10.1016/j.saa.2021.120045
3. Xie L, Zhu G, Shang J, et al. An overview on the biological activity and anti-cancer mechanism ofLovastatin. Cell Signal. 2021; 87:110122. doi: 10.1016/j.cellsig.2021.110122
4. Al-Saman MA, Helmy MA, Abdella A, Wilkins MR, Bobba NA, Mahrous H. Optimization of lovastatin production by Aspergillus terreus ATCC 10020 using solid-state fermentation and its pharmacological applications. Biocatal Agric Biotechnol. 2021; 31:101906. doi: 10.1016/j.bcab.2021.101906
5. El-Bondkly AA, El-Gendi MM, El-Bondkly A. Construction of efficient recombinant strain through genome shuffling in marine endophytic Fusarium sp. ALAA-20 for improvement lovastatin production using agro-industrial wastes. Arab J Sci Eng. 2021; 46(1): 175-90. doi: 10.1007/s13369-020-04925-5
6. Nata NA, Said FM, Shaarani SM, Nawi M, Harun N. Simulation of Lovastatin Production in Solid-State Fermentation via Oil Palm Frond. J Chem Technol Biotechnol. 2021; 7(2):7-10. doi: 10.15282/jcteb.2021.072.6755
7. Oliveira MC, Paulo AJ, Lima CD, de Lima Filho JL, Souza-Motta CM, Vidal EE, Nascimento TP, Marques DD, Porto AL. Lovastatin producing by wild strain of Aspergillus terreus isolated from Brazil. Prep Biochem Biotechnol. 2021; 51(2): 164-72. doi: 10.1080/10826068.2020.1805624
8. Veernala PK, Yugandhar NM. Isolation and Screening of Lovastatin production using fungal isolate from soil samples under solid state fermentation. In Narasimhulu K, Chandran T (eds.), Abstracts of National Conference on Biological, Biochemical, Biomedical, Bioenergy, and Environmental Biotechnology. AJIR Publisher, Birlaumpur, India. 2021: 15.

9. Tsiantas K, Tsiaka T, Koutrotsios G, Siapi E, Zervakis GJ, Kalogeropoulos N, Zoumpoulakis P. On the identification and quantification of ergotormine and lovastatin in various mushroom species: Assets and challenges of different analytical approaches. *Molecules*. 2021; 26(7):1832. doi: 10.3390/molecules26071832

10. Upendra RS, Khandelwal P. Recent advancements in fermentation studies for lovastatin biosynthesis. In Microbial Biotechnology in Food and Health, Academic Press, Cambridge, United States. 2021.

11. Batista BN, Matias RR, Oliveira RL, Albuquerque PM. Hydrolytic enzyme production from açai palm (*Euterpe precatoria*) endophytic fungi and characterization of the amylolytic and cellulolytic extracts. *World J Microbiol Biotechnol*. 2022; 38(2):1-3. doi: 10.1007/s10295-022-03217-w

12. Chen H, Chen J, Qi Y, et al. Endophytic fungus *Cladosporium uncinunissum* DF11, an efficient inducer of tanshinone biosynthesis in *Salvia miltiorrhiza* roots. *Phytochemistry*. 2022; 194:113021. doi: 10.1016/j.phytochem.2021.113021

13. dos Santos IR, Mohamed TA, Borges LL, et al. The global tendency in the research of biological activity in endophytic fungi: a scientometric analysis. *Curr Res Environ Appl Microbil*. 2022; 12(1):1-4. doi: 10.5943/cream/12/1/1

14. Hridoy M, Gorapi M, Hossain Z, et al. Putative Anticancer Compounds from Plant-Derived Endophytic Fungi: A Review. *Molecules*. 2022; 27(1): 296. doi: 10.3390/molecules27010296

15. Macias-Rubalcava ML, Garrido-Santos MY. Phytotoxic compounds from endophytic fungi. *Appl Microbial Biotechnol*. 2022; 106(3):1-20. doi: 10.1007/s00253-022-11773-w

16. Nagarajan K, Ibrahim B, Bawadijki AA, et al. Recent Developments in Metabolomics Studies of Endophytic Fungi. *J Fungi*. 2022; 8(1):28.

17. Ortega HE, Torres-MendozaD, Caballero EZ, Cubillas L. Structurally uncommon secondary metabolites derived from endophytic fungi. *J Fungi*. 2021; 7(7): 570. doi: 10.3390/jf7070570

18. Rigobelo EC, Baron NC. Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. *MycoLOGy*. 2022; 13(1):39-55.

19. Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C. Mevinolin A Highly Potent Competitive Inhibitor of Hydroxymethylglutaryl-Coenzyme A Reductase and a Cholesterol-lowering Agent. *Proc Natl Acad Sci*. 1980; 77(7):3957-3961. doi: 10.1073/pnas.77.7.3957

20. Su YC, Wang JJ, Lin TT, Pan TM. Production of the secondary metabolites γ-aminobutyric acid and monacolin K by *Monascus*. *J Ind Microbiol Biotechnol*. 2003; 30(1): 41-46. doi: 10.1007/s10295-002-0001-5

21. Casas Lopez JL, Sanchez Perez JA, Fernandez Sevilla JM, et al. Production of Lovastatin by *Aspergillus* species: effects of the C:N ratio and the Principal Nutrients on Growth and Metabolite Production. *Enzyme Microb Technol*. 2003; 33(2-3):270-277. doi: 10.1016/S0141-0229(03)00130-3

22. Dragone G, Mussatto SI, Almeida e Silva JB, Teixeira JA. Optimal Fermentation Conditions for Maximizing the Ethanol Production by *Kluyveromyces fragilis* from Cheese Whey Powder. *Biomass Bioenerg*. 2011; 35(5): 1977-1982. doi: 10.1016/j.biombioe.2011.01.045

23. Hajjaj H, Niederberger P, Duboc P. Lovastatin Biosynthesis by *Aspergillus terreus* in a Chemically Defined Medium. *Appl Environ Microbiol*. 2001; 67(6): 2596-2602. doi: 10.1128/AEM.67.6.2596-2602.2001

24. Karthika C, Sharmila G, Muthukumaran C, Krishnan M. Utilization of Whey Powder as an Alternate Carbon Source for Production of Hypcholesterolemic Drug by *Aspergillus terreus* MTCC 1281. *Food Sci Technol*. 2013; 22(5): 1-7. doi: 10.1007/s10068-013-0220-8

25. Pushpa H, Priyata H, Nomita Devi K, Onya N, Vijayalakshmi A, Ramesh DH. Screening of lovastatin (HMG-CoA reductase inhibitor) from edible wild mushrooms. *Curr Res Environ Appl Mycol*. 2016; 6(3): 190-196. doi: 10.5943/cream/63/6

26. OzmiHCI S, Kargi F. Fermentation of Cheese Whey Powder Solution to Ethanol in a Packed-Column Bioreactor: Effects of Feed Sugar Concentration. *J Chem Technol Biotechnol*. 2009; 84(1): 106-111. doi: 10.1002/jctb.2013

27. Chang YN, Lin YC, Lee CC, Liu BL, Tseng YM. Effect of rice-glycerol complex medium on the production of lovastatin by *Monascus* sp. *Folia Microbiol*. 2002; 47:677-684. doi: 10.1016/S0141-0229(02)018671

28. Meng X, Fang Y, Ding M, et al. Developing fungal heterologous expression platforms to explore and improve the production of natural products from fungal biodiversity. *Biotecnol Adv*. 2022; 54:107866. doi: 10.1016/j.biotechad.2021.107866

29. Panda BP, Javed S, Ali M. Optimization of fermentation Parameters for higher lovastatin production in Red mold rice through co-culture of *Monascus purpureus* and *Monascus ruber*. *Food Bioprocess Technol*. 2008; 53: 373-378.

30. Pansuriya RC, Singhal RS. Response surface methodology for optimization of production of lovastatin by solid state fermentation. *Braz J Microbiol*. 2010; 41:164-172. doi: 10.1590/S1517-838220100001000024

31. Pie-Lian WEI, Zhi-nan XU, Pei-Lin CEN. Lovastatin production by *Aspergillus* species in Solid-State fermentation. *J Zheijan Univ*. 2007; 9:1521-1526.

32. Siamak M, Moazami N, Haghighi S, Mohseni F, Mirdamadi S, Bakhtiari M. Screening of lovastatin production by an oleaginous fungus, *Aspergillus terreus* in Solid-State Fermentation. *Environ Appl Microbiol*. 2001; 67(6): 2596-2602. doi: 10.1128/AEM.67.6.2596-2602.2001

33. Srinivasan N, Thangavelu K, Uthandi S. Lovastatin production by an oleaginous fungus, *Aspergillus terreus* KPR12 using sago processing wastewater (SWW). *Microbial Cell Fact*. 2022; 21[1]:1-4. doi: 10.1186/s12934-022-01751-2
34. Bizukojc M, Ledakowicz S. Physiological, morphological and kinetic aspects of lovastatin biosynthesis by \textit{Aspergillus terreus}. \textit{Biotechnol J.} 2009;4:647–664. doi: 10.1002/biot.200800289

35. Mulder KC, Mulinari F, Franco OL, Soares MS, Magalhães BS, Parachin NS. Lovastatin production: from molecular basis to industrial process optimization. \textit{Biotechnol Adv.} 2015;33:648–665. doi: 10.1016/j.biotechadv.2015.04.001

36. López JC, Pérez JS, Sevilla JF, Fernández FA, Grima EM, Chisti Y. Production of lovastatin by \textit{Aspergillus terreus}: effects of the C:N ratio and the principal nutrients on growth and metabolite production. \textit{Enzyme Microb Technol.} 2003;33:270–277. doi: 10.1016/S0141-0229(03)00130-3

37. Jaivel N, Marimuthu P. Isolation and screening of lovastatin producing microorganisms. \textit{Int J Eng Sci Technol.} 2010;2:2607–2611.

38. Pecyna M, Bizukojc M. Lovastatin biosynthesis by \textit{Aspergillus terreus} with the simultaneous use of lactose and glycerol in a discontinuous fed-batch culture. \textit{Biotechnol J.} 2011;151:77–86. doi: 10.1016/j.biotechadv.2010.10.079

39. Sridevi B, Charya MAS. Isolation, identification and screening of potential cellulase-free xylanase producing fungi. \textit{Afr J Biotechnol.} 2011;10:4624–4630.

40. Mahmoud OA, Abdel Hadi SY. Extraction and Purification of Lovastatin from the Edible Mushroom \textit{Laetiporus sulphureus} and its Antioxidant Activity. \textit{Egypt J Bot.} 2022; 62(1):169-75.