Biosynthesis of Lovastatin by Gamma Irradiated Aspergillus Terreus

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ABSTRACT

Lovastatin (C₂₄H₃₆O₅, Mevinolin, Monacolin K and Mevacor®) is the first compound of its kind to become available for treatment of hypercholesterolemia. This fungal secondary metabolite is produced by Aspergillus, Monascus and Penicillium species, via the polyketide synthase (PKSs). The role of hypercholesterolemia as a risk factor for atherosclerosis, and ischemic heart disease was indicated by the clinical, epidemiologic and pathologic studies.

In the present study, gamma irradiated of selected highly lovastatin producer Aspergillus terreus isolate (A. terreus S3γ8) was employed for lovastatin production in submerged fermentation (SmF) conditions. Different fermentation parameters including: incubation period, initial pH of the medium, temperature, different carbon and nitrogen sources, type of inoculum and agitation; were carried out under SmF conditions to enhance the lovastatin production. The maximum lovastatin production (547.33 mg/l) was achieved at initial pH 6, incubation temperature 30 °C, agitation rate 150 rpm, 4% soluble starch and 0.3% yeast extract as carbon and nitrogen sources, respectively, after 8 days when the production medium (which containing bio-elements: KH₂PO₄, MgSO₄ and MnSO₄) was inoculated with 48 h age from seed culture inoculum.

KEYWORDS

Aspergillus terreus., Lovastatin, Biosynthesis, Gamma radiation.
INTRODUCTION

World Health organization (WHO) estimated till 2015, Non communicable diseases (NCDs) have been caused about 40 million deaths. It was reported that globally cardiovascular diseases (CVDs) are the major cause of these deaths than any other cause due to NCDs deaths. Among four major death causes of NCDs, CVDs accounts 45% (about 17.7 million deaths. Further, it was reported that low and middle income countries are affected more than ¾ of CVD deaths than others (WHO, 2017). This is related to high levels of cholesterol in plasma, since hypercholesterolemia is primary risk factor of atherosclerosis and coronary artery disease (Barrios-Gonzalez and Miranda, 2010). It has also been reported that high cholesterol level increases the risk of several nervous system diseases like dementia/Alzheimer’s disease Ischemic heart stroke (IHS). Further, hypercholesterolemia stimulates the chances of diabetes development, obesity and certain types of cancers (Munir et al., 2018).

Generally, only one-third of the total body cholesterol is diet-derived; while two-thirds are synthesized by the liver and, to a lesser extent by other organs (Furberg, 1999). For this reason, control of cholesterol by inhibiting its biosynthesis is an important strategy to lower cholesterol levels in blood.

Statins are a group of drugs that selectively inhibit the enzyme 3-hydroxy-3- methylglutaryl-coenzyme A (HMG-CoA) reductase, the regulatory and rate-limiting enzyme in cholesterol biosynthesis (Bizukojc and Ledakowicz, 2015). In this way, these compounds lower cholesterol; particularly low density lipoprotein (LDL) or low den sity cholesterol (“bad cholesterol”); while slightly increasing high-density lipoprotein cholesterol (“good cholesterol”), thus, preventing plaque buildup inside the arteries. Moreover, statins have emerged at the forefront of preventive drugs for cardiovascular disease because of a substantial clinical trial database demonstrating that statins reduce the risk for coronary artery disease morbidity and death across a broad range of at-risk patient cohorts. A different group of studies have shown that statin therapy has biological effects beyond the level of LDL cholesterol. These new studies have discovered numerous new biological (pharmacological) activities of statins; representing potential application in diseases like cancer, Alzheimer’s dementia and age-related bone loss. Statins are lovastatin and compactin, while pravastatin is derived from the latter by biotransformation. Simvasatin, the second leading statin in the market, is a lovastatin semisynthetic derivative.

Lovastatin (C₂₄H₃₆O₅, also known as mevinolin, monacolin K, Mevacor) is a potent competitive inhibitor of HMG-CoA reductase. It is active not only in vitro to inhibit cholesterol biosynthesis but also in vivo to lower plasma cholesterol level in humans and animals (Chang et al., 2002 and Bizukojc and Ledakowicz, 2015), and is thereby effective in the therapy of hypercholesterolemia. Lovastatin was the first hypcholesterolemic drug to be approved by the United States Food and Drug Administration (Manzoni and Rollini, 2002). It has also been indicated as a potential therapeutic agent for the treatment of various types of tumors because of its capability to suppress tumor growth in vivo through inhibition the synthesis of nonsterol isoprenoid compounds (Chang et al., 2002).

Different types of filamentous fungi including Penicillium species Aspergillus species, and Monascus species have higher capability for lovastatin production through fermentation technique. It was also reported that species of Scopolaripiosis, Paecilomyces, Doratomyces, Pleurotus,Trichoderma, Phytilium, Phoma, Gymnoascus and Hypomyces have also potential to produce lovastatin during the
course of fermentation ((Munir et al., 2018 and Iewkittayakorn et al., 2020).

The traditional fermentation process involving SSF is labour-intensive, time-consuming and requires large cultivation areas, therefore the utilization of SmF technique for the production of fungal secondary metabolites has been studied to overcome the problems of space, scale-up and process control of SSF (Lai et al., 2005). It is well known that the culture medium has a significant influence on the biosynthesis of lovastatin and its rate of production. Selection and composition optimization of an optimum medium is therefore important for establishing a process for producing lovastatin (Li et al., 2011; Suwannarat et al., 2019 and Subhan et al., 2020).

Although mutation breeding is an effective method of improving the production of lovastatin, and many mutagenic techniques have been used to improve the productivity of A. terreus, to our knowledge, there have been nil reports of using gamma irradiation to induce high-yield lovastatin mutants of A. terreus (Gu et al., 2008 and Li et al., 2011).

The objective of this study is to investigate lovastatin production by gamma irradiated Aspergillus terreus (local isolated strain) in submerged (SmF) conditions.

MATERIALS AND METHODS

Microorganisms and inoculum preparation:

The Aspergillus terreus isolates used in this study were obtained from Industrial Microbiology Laboratory, National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The isolates were cultured on Potato Dextrose Agar (PDA, Oxoid, 2006) slants at 25°C for 10 days, and then stored at 4°C, and sub cultured every 3 months. A suspension of spores was prepared by washing PDA slants cultures with a sterile saline solution of 0.1% Tween-80. The spore concentration was determined by direct plate counts of spores in suspension and adjusted to 10⁶ spores/ml by diluting it suitably. A spore suspension of this concentration was used as inoculum.

Screening of lovastatin producer isolates on broth medium:

The screening for lovastatin production was carried out with nine isolates of A. terreus. The screening was carried out in Erlenmeyer flasks (250 ml) each containing 50 ml of different sterile production media (PMI, Manzoni et al., 1998; PMII, Su et al., 2003; PMIII, Chang et al., 2002; PMIV, Manzoni et al., 1998 and PMV, Sayyad et al. 2007). The flasks (initial pH 6) were inoculated with 1 ml spore suspension (10⁶ CFU/ml) from each tested isolate. The inoculated flasks were incubated at 30 °C at 6 and 12 days at 150 rpm. After incubation period, the fermented culture media were extracted by ethyl acetate and the organic phase was used to determine the lovastatin content. Also, microbial growth was determined by drying the biomass on filter paper for 24h at 65°C to a constant weight. The highly producer lovastatin isolate was selected and used for further investigation in this study.

Induction of active isolates:

Three milliter of highly producer lovastatin A. terreus spores (10⁶ spores / ml) were exposed to gamma radiation at dose 4 kGy (sub lethal dose) which kill about 99.99% of survival spores. Irradiation was carried out at the National Center for Radiation Research and Technology (NCRRT), using ⁶⁰Co gamma irradiation source of gamma chamber (4000A) with a dose rate 1 kGy /52 min at the time of the experiment.

The irradiated spores were grown for 4 days on PDA plates and the purified survival irradiated colonies (26 colonies) were picked up and grown on lactose-yeast extract agar medium for 7 days at
28°C. After incubation the rapid screening method (Vilches Ferron et al., 2005) was used for isolating lovastatin overproducing irradiated isolate of *A. terreus*. Also, the higher lovastatin producer irradiated isolates were screened for lovastatin production in broth suitable PM.

**Lovastatin production parameters**

The highly irradiated lovastatin producer *A. terreus* isolate was selected for the investigation of some conditions affecting lovastatin production from the best selected production medium in flask batch cultures. Batch fermentations were carried out in 250 ml Erlenmeyer flasks containing 50 ml of PM. A set of experiments was performed at different period (2-12 days), pH (4-8) temperature (20-35 °C), eight carbon sources (30 g/l), eight nitrogen sources (3.86 g/l), type of inoculum (spore suspension, seed culture and mycelium disc) and aeration to investigate the effect of these parameters on the lovastatin production. For preparation of seed culture 1.0 ml of fungal spore suspension (2 x 10⁷ CFU/ml) was inoculated in seed culture medium (Yeast Malt Extract, Oxoid, 2006) and incubated under shaking (150 rpm) at 30 °C for 2 days. After 2 days, 5 ml of the fungal growth was used as inoculums (10v/v). For preparation of mycelium fungal growth, the fungus was inoculated on PDA plates at 28 °C for 7 days. After that the fungal growth was cut into 1 cm discs by sterile cork porer, and one disc was used as inoculum.

**Analytical Methods:**

The lovastatin in the fermented culture was determined according to methods described by Hajko et al. (1998); Kumar et al. (2000) and Li et al. (2011) with some modifications. The fermentation broth was adjusted to pH 3.0 using HCl (36%), after which an equal volume of ethyl acetate was then added. After shaking at 180 rpm for 12 h at ambient temperature, the fermentation broth and the mycelium pellets were filtered through preweighed membrane filters and the residual biomass was washed three times with distilled water. The biomass was determined by gravimetric analysis after the mycelium pellets were dried at 65 °C to a constant weight. The organic and aqueous phases from the filtrate were separated in a separating funnel. The organic phase was dried under vacuum at 45°C. The dried residue was dissolved in 5 ml of 75% ethanol and used to determine the lovastatin content against known concentration of pure lovastatin. Lovastatin contents were determined by the dual-wavelength UV spectrophotometry method. To verify the efficiency of this method, extraction of blank (fermented culture without inoculation) and known concentration (20 mg) of pureLovastatin (as internal standard) in fermented culture was carried out at the same conditions. Verification of lovastatin contents were determined by through scanning between 190-1100 nm by using UV/Visible Spectrophotometer (type Helios Gamma) to determine the maximum absorbance (λ max) wavelength of the produced lovastatin and the standard concentration (20 mg) of pure lovastatin. The λ max showed maximum reading was fixed and used for determining of extracted produced lovastatin in this study.

Residual sugar was measured by the phenol sulphuric acid method (Southgate, 1976) using glucose as standard.

**RESULT AND DISCUSSION**

**Screening of some isolates for lovastatin production**

Nine *A. terreus* isolates were screened in specific lovastatin production broth media (table 1). It was found that 7 isolates of *A. terreus* have the ability to produce lovastatin. The highest lovastatin concentration (64 mg/l) was recorded by *A. terreus* isolate number 3 (*A. terreus* S3) after 12 days from PMII medium. Manzoni, et al. (1999) found that of
all Monascus and Aspergillus strains investigated for statins production, *M. paxii* AM12M, an isolated spontaneous mutant, yielded 127 mg lovastatin/l.

Culture media for microbial lovastatin production are very diverse, ranging from defined compositions to natural ones. The results of lovastatin produced by *A. terreus* in this study indicated that the PMII medium was the best medium for lovastatin production. The production medium PMII consists of (g/l): Dextrose, 30; NH$_4$Cl, 3.86; KH$_2$PO$_4$, MgSO$_4$·7H$_2$O, 0.86; MnSO$_4$·4H$_2$O, 0.19 (Su et al., 2003). The main carbon and nitrogen sources in this medium were dextrose (30 g/l) and NH$_4$Cl (3.86 g/l), respectively, thus confirming the importance of this medium for high lovastatin yields by *M. purpureus* MTTCC 369 (Sayyad et al., 2007). On other hand Chang, et al. (2002); Sayyad, et al. (2007) and Li, et al. (2011) observed that the components of the complex culture media used for Monascus and Aspergillus lovastatin production include several sugars (most commonly glucose, lactose and glycerol) organic nitrogen sources (peptones and yeast extract) or inorganic nitrogen (ammonium and nitrates).

### Table (1) : Fermentation yields (mg/l) of lovastatin by different *A. terreus* isolates from different media after 6 and 12 days. (growth conditions: pH,6; temp. 30°C; agitation rate,150 rpm).

| A. terreus isolates | Different culture media | PMI 6 | PMI 12 | PMII 6 | PMII 12 | PMIII 6 | PMIII 12 | PMIV 6 | PMIV 12 | PMV 6 | PMV 12 |
|---------------------|-------------------------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| S1                  | PMI                     |   14  |   38   |   11   |   35   |   20   |   48   |   16   |   37   |   12   |   26   |
| S2                  | PMI                     |   18  |   46   |   16   |   47   |   14   |   39   |   12   |   34   |   11   |   31   |
| S3                  | PMII                    |   22  |   56   |   28   |   64   |   20   |   58   |   18   |   43   |   17   |   52   |
| S4                  | PMII                    | ND    | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     |
| S5                  | PMII                    |   15  |   47   |   20   |   53   |   19   |   46   |   14   |   39   |   14   |   35   |
| S6                  | PMII                    |   12  |   35   |   10   |   32   |   17   |   44   |   15   |   40   |   13   |   42   |
| S7                  | PMII                    |   13  |   30   |   15   |   36   |   14   |   32   |   11   |   24   |   16   |   40   |
| S8                  | PMII                    | ND    | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     | ND     |
| S9                  | PMII                    |   12  |   36   |   9    |   22   |   16   |   45   |   11   |   36   |    8   |   20   |

*ND = Not detected*

Comparing the results of the lovastatin produced by the 7 producer *A. terreus* strains indicated that *A. terreus* S3 was the most efficient strain; consequently it was selected to carry out further investigations.

### Effect of gamma radiation

In a previous study a rapid screening method of *A. terreus* mutants for overproduction of lovastatin was reported (Vilches Ferron et al., 2005). The authors investigated that lovastatin caused growth inhibition of *C. albicans* in submerged cultures and on solid medium, and when lovastatin was placed on the agar surface using a paper disk, inhibition zones were obtained on plates of *C. albicans*.

In the present study, out of 26 survival colonies of *A. terreus* S3, irradiated at dose 4 kGy, only 9 gave lovastatin titres higher than that of the parent culture.
The highest titre, inhibition zone diameter (18 mm), obtained with the active irradiated A. terreus S3γ8, was three times the lovastatin production level of the original culture (6 mm, fig.1).

From the present data, it is clear that the active irradiated A. terreus S3 γ8 isolate produced an over-production of lovastatin and used for further studies. A detailed study of lovastatin production by A. terreus S3γ8 in SmF under various conditions were carried out to obtain a clear picture of the process conditions conducive to the production of higher amounts of lovastatin by this active irradiated isolate.

**Influence of incubation period**

The present results showed that maximum lovastatin secretion by gamma irradiated active isolate A. terreus S3γ8 (184.66 mg/l) was obtained after 8 d of incubation and then decline (table. 2). Lai, *et al. (2007)* found that after the stationary phase of growth, the maximum lovastatin production by A. terreus ATCC 20542 was 873 mg/l on day 10. Also, 10 days were recorded as the best fermentation time for the maximum lovastatin production by A.terreus ((Lai *et al., 2003* and Gupta *et al., 2009)*). On the other hand, the M. paxii AM12M fermentation profile showed that, at 16 days there was already an appreciable yield of lovastatin (117 mg/l), after which it slowly increased to reach 127 mg/l at 21 days (Manzoni *et al., 1999*). Decline of lovastatin production after 10 d was related to the insufficient availability of both carbon and nitrogen sources and/or increasing the broth viscosity at the latter stages of fermentation (12-14d) due to the built up of biomass concentration (Kumar *et al., 2000* and Dominguez-Espinosa and Webb, 2003).

**Table (2) : Time course of lovastatin production by A. terreus S3γ8 grown on production medium PMII.**

| Time/day | Consumed sugar (g/I) | Biomass (g/I) | Lovastatin (mg/I) * | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin productivity (mg/I/h) |
|----------|----------------------|--------------|---------------------|--------------------------|-----------------------------|-------------------------------|
| 2        | 6.52                 | 0.75         | 7.2 ±0.67           | 0.12                     | 1.10                        | 0.15                          |
| 4        | 13.41                | 2.12         | 33.93±1.67          | 0.16                     | 2.53                        | 0.35                          |
| 6        | 17.23                | 4.30         | 91.33± 2.03         | 0.25                     | 5.30                        | 0.63                          |
| 8        | 21.82                | 5.92         | 184.66±4.33**       | 0.27                     | 8.50                        | 0.97                          |
| 10       | 25.24                | 6.31         | 136±2.65            | 0.24                     | 5.41                        | 0.61                          |
| 12       | 28.10                | 6.14         | 104.33±3.28         | 0.22                     | 3.71                        | 0.40                          |

* Mean ± SE  
** Significant from all values (P < 0.01)

**Effect of initial pH**

Hydrogen ion concentration (pH) of the medium is considered one of the most important factors, which not only affected the growth of microorganisms but also has great influence on their physiological activity. In the present work, it was found that the maximum lovasatin (205.66 mg/l) production by A. terreus S3γ8 was obtained at pH 6 (table 3). In agreement, Sayyad, *et al. (2007)*; Kumar, *et al. (2000)*; Chang, *et al. (2002)*; Jia, *et al. (2009)* and Li, *et al. (2011)* reported that the optimum pH for lovastatin production by various Monascus and As-
Biosynthesis of Lovastatin by Gamma Irradiated *Aspergillus terreus* spp. was obtained at pH 5-6.5. On the other hand, Lai *et al.* (2005) found that pH in the process of lovasatin production by *A. terreus* in SmF does not play a significant role, as it usually remains around neutral, and does not require to be adjusted during the process.

**Table (3)**: Effect of pH values on lovasatin production by *A. terreus* S3γ8 grown on PMII.

| Initial pH | Consumed sugar (g/I) | Biomass (g/I) | Lovastatin (mg/I) * | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin Productivity (mg/I/h) |
|------------|----------------------|---------------|---------------------|--------------------------|-----------------------------|----------------------------------|
| 4          | 26.34                | 5.62          | 146.66 ±3.49        | 0.21                     | 5.61                        | 0.80                             |
| 5          | 28.52                | 6.33          | 183±3.79            | 0.22                     | 6.42                        | 0.95                             |
| 6          | 28.13                | 5.90          | 205.66±4.63**       | 0.21                     | 7.32                        | 1.10                             |
| 7          | 22.42                | 4.25          | 71.33± 3.53         | 0.19                     | 3.20                        | 0.40                             |
| 8          | 13.25                | 2.84          | 26±2.31             | 0.21                     | 1.96                        | 0.14                             |

* Mean ± SE  
** Significant from all values (P < 0.01)

**Effect of incubation temperature**

Temperature is one of the most important parameters regulating the activity of microorganisms in natural environments. Generally, there is an optimal temperature for the activity of enzymes produced by different microorganisms which responsible for the biosynthesis or degradation of compounds. This optimal temperature may be similar or different from the optimal temperature of the microbial growth. In this study, it was observed that incubation temperature 30°C was the optimum temperature for maximum lovasatin (198.33 mg/I) production by *A. terreus* S3γ8 (table 4). Similarly, Kumar, *et al.* (2000); Jia, *et al.* (2009) and Azeem, *et al.* (2018) found that the optimum temperature for lovasatin production by *A. terreus* was 28°C. Also, Sayyad, *et al.* (2007) and Suwannarat, *et al.* (2019) reported that the optimum temperature for lovasatin production by *M. purpureus* MTCC 369 and *A. terreus* was 30 and 25°C, respectively. Temperature influences the response of microorganisms directly by its effect on growth rate, enzyme activity, cell composition and nutritional requirements.

**Table (4)**: Effect of incubation temperature on lovasatin production by *A. terreus* S3γ8 grown on PMII.

| Temp (°C) | Consumed sugar (g/I) | Biomass (g/I) | Lovastatin (mg/I) * | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin Productivity (mg/I/h) |
|-----------|----------------------|---------------|---------------------|--------------------------|-----------------------------|----------------------------------|
| 20        | 24.82                | 4.22          | 115.33±3.76         | 0.17                     | 4.70                        | 0.60                             |
| 25        | 27.31                | 6.14          | 171±3.68            | 0.22                     | 6.30                        | 0.90                             |
| 30        | 28.24                | 5.83          | 198.33± 5.04**      | 0.21                     | 7.02                        | 1.03                             |
| 35        | 21.73                | 3.62          | 89.33±3.53          | 0.17                     | 4.11                        | 0.51                             |

* Mean ± SE  
** Significant from all values (P < 0.01)
**Effect of carbon sources**

Carbon sources serve three different functions within the microbial cell forming lovastatin; carbon source for biomass synthesis, an energy source for biosynthesis and cell maintenance and carbon source for lovastatin production. It was found that lovastatin production by the *A. terreus* S3γ8 strain, under investigation, are highly influenced by different type of carbon sources. Starch was the best carbon source for lovastatin (263.33 mg/l) production in this study (table 5). The volumetric production of *A. terreus* lovastatin in submerged media is higher with lactose (Casas Lopez et al., 2003 and Lai et al., 2007). Li, et al. (2011) observed that glycerol and soluble starch was the best carbon sources for production of lovastatin by mutant of *A. terreus* CA99.

Lovastatin synthesis in this study was closely related to the amount of starch. The starch concentration 40 g/l was the optimum concentration to maximum lovastatin (328.33 mg/l) production and increasing the starch concentration above 40 g/l caused inhibition in the lovastatin production, in this study (table 6). This result was also confirmed by Li, et al. (2011) who reported that glycerol and soluble starch (30 g/l) in the chemically defined medium gave the highest yield of lovastatin produced by mutant of *A. terreus* CA99. The increased amount of starch in the medium caused an increase in the medium viscosity, which led to poor medium aeration of the culture and consequently lovastatin production was minimal.

**Table (5) : Effect of different carbon sources on lovastatin production by A. terreus S3γ8 grown on PMII.**

| Sugar sources (30g/l) | Consumed sugar (g/I) | Biomass (g/I) | Lovastatin (mg/I) * | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin Productivity (mg/I/h) |
|-----------------------|---------------------|--------------|---------------------|---------------------------|-----------------------------|--------------------------------|
| Dextrose «control»     | 28.24               | 5.92         | 193.66 ±6.12        | 0.21                      | 6.91                        | 1.01                           |
| Sucrose               | 26.52               | 6.13         | 140.66 ±3.49        | 0.23                      | 5.30                        | 0.73                           |
| Fructose              | 17.30               | 4.22         | 84.33 ±2.33         | 0.24                      | 4.87                        | 0.44                           |
| Starch                | 28.64               | 5.67         | 263.33±5.24**       | 0.20                      | 9.20                        | 1.40                           |
| Maltose               | 18.50               | 4.41         | 96.66±3.49          | 0.24                      | 5.23                        | 0.50                           |
| Lactose               | 25.42               | 6.30         | 231 ±3.79           | 0.25                      | 9.11                        | 1.20                           |
| Manitol               | 13.15               | 3.72         | 44.33 ±1.76         | 0.28                      | 3.37                        | 0.23                           |
| Xylose                | 12.6                | 3.31         | 33±2.10             | 0.26                      | 2.62                        | 0.20                           |

* Mean ± SE
** Significant from all values (P < 0.01)

**Table (6) : Effect of different starch concentrations on lovastatin production by A. terreus S3γ8 grown on PMII.**

| Starch Conc. (g/I) | Consumed sugar (g/I) | Biomass (g/I) | Lovastatin (mg/I) * | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin Productivity (mg/I/h) |
|-------------------|---------------------|--------------|---------------------|---------------------------|-----------------------------|--------------------------------|
| 10                | 9.62                | 2.84         | 25.33±2.03          | 0.31                      | 2.63                        | 0.13                           |
| 20                | 19.14               | 4.15         | 83±3.22            | 0.22                      | 4.34                        | 0.43                           |
| 30                | 28.50               | 5.61         | 261.66±5.55        | 0.21                      | 9.20                        | 1.40                           |
| 40                | 36.22               | 6.80         | 328.33±4.91**      | 0.21                      | 9.10                        | 1.71                           |
| 50                | 41.83               | 7.52         | 284.66±4.33        | 0.20                      | 6.81                        | 1.50                           |
| 60                | 52.61               | 8.40         | 211.66±3.76        | 0.21                      | 4.02                        | 1.10                           |

* Mean ± SE
** Significant from all values (P < 0.01)
**Effect of nitrogen sources**

Nitrogen sources appear to be one of the most effective factors for the microbial lovastatin production (Casas Lopez et al., 2003). Studies on several organic and inorganic nitrogen sources showed that yeast extract was the best nitrogen source for lovastatin (415.33 mg/l) production by A. terreus S3γ8 strain under investigation (table 7). Organic nitrogen sources (peptone and yeast extract) and corn meal were recorded as the best nitrogen sources for production of microbial lovastatin (Manzoni et al., 1998; Casas Lopez et al., 2003; Li et al., 2011 and Azeem et al., 2018).

The development of a nutrient strategy is crucial to the success of the fermentation process. Lovastatin production needs to be balanced to avoid incomplete secretion of it. This may be achieved by balancing the carbon/nitrogen ratio. In the present investigation, the maximum lovastatin production by A. terreus S3γ8 (442.66 mg/l) was obtained at 3 g/l yeast extract concentration (table 8). Under the nitrogen sufficient condition, Manzoni, et al. (1998); Kumar, et al. (2000); Chang, et al. (2002); Sayyad, et al. (2007) and Li, et al. (2011) found that the Aspergillus and Monascus spp. produced the highest lovastatin concentrations.

**Effect of inoculum type**

Despite the importance of inoculum development little work has been published that addresses the problem of inoculum optimization (DeTilly et al., 1983). For lovastatin production by fungi in SmF, three main inoculation types been used: spore suspension, mycelial mat and seed culture (Novak et al., 1997; Casas Lopez et al., 2003 and Lai et al., 2003). The present results showed that seed culture inoculum 48h age of A. terreus S3γ8 at density 10% v/v was the best inoculum preparation for theLovastatin (545.33 mg/l) production (table 9). Sayyad, et al. (2007) used seed culture inoculum 48h age at density 10% (v/v) for M. purpureus MTCC 369 lovastatin production. On the other hand 5% (v/v) of seed culture inoculum (4 old days) was used as inoculum for M. ruber ATCC 18199 lovastatin production (Chang et al., 2002).

**Table (7)**: Effect of different nitrogen sources on lovastatin production by A. terreus S3γ8 grown on PMII.

| Nitrogen sources (3.86 g/l) | Consumed sugar (g/l) | Biomass (g/l) | Lovastatin (mg/l) | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin Productivity (mg/l/h) |
|---------------------------|----------------------|--------------|------------------|--------------------------|-----------------------------|---------------------------------|
| NH₄Cl “control”           | 36.52                | 6.83         | 334±4.73         | 0.19                     | 9.15                        | 1.74                            |
| NaNO₃                    | 33.62                | 6.13         | 241.33±5.24      | 0.18                     | 7.20                        | 1.30                            |
| KNO₃                     | 28.83                | 5.42         | 193.33±5.24      | 0.21                     | 6.71                        | 1.01                            |
| (NH₄)₂SO₄                | 35.40                | 7.22         | 353.33±5.81      | 0.20                     | 9.98                        | 1.84                            |
| Mycological peptone      | 37.14                | 7.60         | 382.33±7.31      | 0.20                     | 10.30                       | 2.10                            |
| Yeast extract            | 36.81                | 6.62         | 415.33±6.64**    | 0.21                     | 11.30                       | 2.20                            |
| Tryptone                 | 34.20                | 7.41         | 226.33±4.91      | 0.22                     | 6.62                        | 1.21                            |
| Beef extract             | 35.22                | 7.13         | 261.66±6.12      | 0.20                     | 7.43                        | 1.40                            |

* Mean ± SE
** Significant from all values (P < 0.01)
It has been reported that agitation (oxygen transfer) is one of the key parameters for the process optimization and scale-up of lovastatin production by fungi (Su et al., 2003 and Sayyad et al., 2007). It is apparent from the result that the productivity of *A. terreus* S3γ8 lovastatin varies considerably during static and shaking conditions (table 10). More lovastatin was found to be produced in shaking conditions as compared to static one, and 150 rpm was the best agitation speed for lovastatin (547.33 mg/l) production, in this study, but a further increase (200 rpm) was not associated with a better production. Lai et al. (2003) and Azeem et al. (2018) mentioned that the production of *A. terreus* lovastatin have mainly based on agitation speed on 200 rpm. In contrast agitation rate (60 rpm) were used for maximize several Monascus and Aspergillus spp. statins production (Manzoni et al., 1999). Morphological changes and destruction of the pellets were associated with the high agitation rate (in this study) resulting in high shear conditions. These morphological changes are certainly related to physiological changes resulting in a lower production of secondary metabolites (Hajjaj et al., 1999).

### Table (8): Effect of yeast extract concentration on lovastatin production by *A. terreus* S3γ8 grown on PMII.

| Yeast extract conc. (g/l) | Consumed sugar (g/l) | Biomass (g/l) | Lovastatin (mg/l) * | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin Productivity (mg/l/h) |
|-------------------------|----------------------|--------------|---------------------|--------------------------|----------------------------|-------------------------------|
| 1                       | 31.50                | 5.21         | 274±4.36            | 0.17                     | 8.71                       | 1.43                          |
| 2                       | 35.82                | 6.40         | 382.33±7.31         | 0.18                     | 10.70                      | 2.11                          |
| 3                       | 36.84                | 6.62         | 442.66±6.94**       | 0.21                     | 12.01                      | 2.30                          |
| 4                       | 36.60                | 6.51         | 394±6.43            | 0.21                     | 10.81                      | 2.10                          |
| 5                       | 31.42                | 5.52         | 234±4.36            | 0.18                     | 7.50                       | 1.22                          |
| 6                       | 25.30                | 4.11         | 179±3.80            | 0.16                     | 7.11                       | 0.93                          |

* Mean ± SE
** Significant from all values (P < 0.01)

### Table (9): Effect of type of *A. terreus* S3γ8 inocula on lovastatin production from optimized PMII (OPMII).

| Inoculum type | Consumed sugar, (g/l) | Biomass (g/l) | Lovastatin (mg/l) * | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin Productivity (mg/l/h) |
|---------------|-----------------------|--------------|---------------------|--------------------------|----------------------------|-------------------------------|
| Spore suspension, 1% (Control) | 36.80 | 6.61 | 482.66±7.62 | 0.18 | 13.12 | 2.51 |
| Seed culture (Age, 48 h; 10%) | 37.52 | 6.80 | 545.33±7.51** | 0.18 | 14.53 | 2.84 |
| Mycelium Disc 1 cm, 7 days old | 35.41 | 6.22 | 346.33±6.64 | 0.19 | 9.80 | 1.80 |

* Mean ± SE
** Significant from all values (P < 0.01)
**Table (10): Effect of agitation on lovastatin production by A. terreus S3γ8 on OPMII.**

| Agitation speed (rpm) | Consumed sugar (g/L) | Biomass (g/L) | Lovastatin yield (mg/L) | Biomass yield coefficient | Lovastatin yield coefficient | Lovastatin Productivity (mg/L/h) |
|-----------------------|----------------------|---------------|-------------------------|--------------------------|-----------------------------|---------------------------------|
| 0 (static)            | 25.20                | 7.12          | 113.66 ±4.10            | 0.28                     | 4.51                        | 0.60                            |
| 50                    | 30.41                | 5.80          | 210±4.62                | 0.20                     | 7.01                        | 1.10                            |
| 100                   | 32.53                | 6.22          | 293.33±5.24             | 0.19                     | 9.02                        | 1.53                            |
| 150                   | 37.40                | 6.81          | 547.33±5.81**           | 0.18                     | 14.63                       | 2.86                            |
| 200                   | 37.12                | 6.52          | 327±5.51                | 0.17                     | 8.81                        | 1.70                            |
| 250                   | 35.20                | 5.30          | 240±4.62                | 0.15                     | 7.02                        | 1.25                            |

* Mean ± SE

** Significant from all values (P < 0.01)

In conclusion, the social progress and the rise-up of human living standards, more and more attention has been paid to the health cares. Lovastatin (C₂₄H₃₆O₅) is the first compound of its kind to become available for treatment of hypercholesterolemia. This fungal secondary metabolite is produced by *Aspergillus terreus*, *Monascus* species and *Penicillium* species, via the polyketide synthase (PKSs).

The present work has been devoted to studying the effect of different parameters on lovastatin production by the local isolated strain of *A. terreus*. The production of lovastatin by gamma irradiated isolate (*A. terreus S3γ8*) in SmF is not a novel idea, but, limited information is available on culture parameters influencing lovastatin production by this method. Therefore, this study mainly investigated how much the physical and nutritional fermentation parameters affect the yield of lovastatin production by *A. terreus S3γ8*. However, scales up studies are still necessary to further optimize the proposed process and to evaluate its techno-economical feasibility.

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