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Biosynthesis of Lovastatin by Gamma Irradiated *Aspergillus Terreus*

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ABSTRACT

Lovastatin ($C_{24}H_{36}O_5$, 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: KH_2PO_4, $MgSO_4$ and $MnSO_4$) was inoculated with 48 h age from seed culture inoculum.

KEYWORDS

Aspergillus terreus., Lovastatin, Biosynthesis, Gamma radiation.

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.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. Simvastatin, the second leading statin in the market, is a lovastatin semisynthetic derivative.

Lovastatin ($C_{24}H_{36}O_5$, 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 hypocholesterolemic drug to be approved by the United States Food and Drug Administration (**Manzoni and Rollini, 2002**). It has also been indicated as 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 *Scopolariopsis, Paecilomyces, Doratomyces, Pleurotus,Trichoderma, Phythium, 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 10^6 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 (10^6 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 (10^6 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 ^{60}Co 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 10^7 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 filtrated 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 pure lovastatin (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 lovasatatin. 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_4Cl, 3.86; KH_2PO_4; $MgSO_4.7H_2O$, 0.86; $MnSO_4.4H_2O$, 0.19 (**Su *et al.*, 2003).** The main carbon and nitrogen sources in this medium were dextrose (30 g/l) and NH_4Cl (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).*

Different culture media / *A. terreus* isolates	PMI		PMII		PMIII		PMIV		PMV	
	6	12	6	12	6	12	6	12	6	12
S1	14	38	11	35	20	48	16	37	12	26
S2	18	46	16	47	14	39	12	34	11	31
S3	22	56	28	64	20	58	18	43	17	52
S4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S5	15	47	20	53	19	46	14	39	14	35
S6	12	35	10	32	17	44	15	40	13	42
S7	13	30	15	36	14	32	11	24	16	40
S8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S9	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* S3was 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

(fig. 1). 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 overproduction 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.

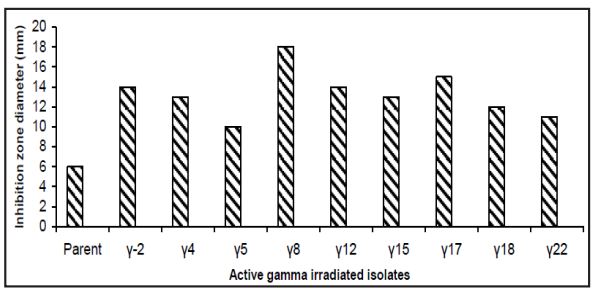

Fig. (1): Inhibition zone diameter on plates of *C. albicans* vs. lovastatin produced by some active gamma irradiated *A. terreus* S3 isolates.

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**). 0n 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 lovastatin (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-*

pergillus spp. was obtained at pH 5-6.5. On the other hand, **Lai *et al.* (2005)** found that pH in the process of lovastatin 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 lovastatin production by A. terreus S3γ8 grown on PMII.*

Initial pH	Consumed sugar (g/l)	Biomass (g/l)	Lovastatin (mg/l) *	Biomass yield coefficient	Lovastatin yield coefficient	Lovastatin Productivity (mg/l/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 lovastatin (198.33 mg/l) 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 lovastatin production by *A. terreus* was 28°C. Also, **Sayyad, *et al.* (2007)** and **Suwannarat, *et al.* (2019)** reported that the optimum temperature for lovastatin 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 lovastatin production by A. terreus S3γ8 grown on PMII.*

Temp (°C)	Consumed sugar (g/l)	Biomas (g/l)	Lovastatin (mg/l) *	Biomass yield coefficient	Lovastatin yield coefficient	Lovastatin Productivity (mg/l/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. te*rreus 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/I)	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 need 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 pr\oblem 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 the lovastatin (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/I)	Consumed sugar (g/I)	Biomass (g/I)	Lovastatin (mg/I) *	Biomass yield coefficient	Lovastatin yield coefficient	Lovastatin Productivity (mg/I/h)
NH_4Cl "control"	36.52	6.83	334±4.73	0.19	9.15	1.74
$NaNO_3$	33.62	6.13	241.33±5.24	0.18	7.20	1.30
KNO_3	28.83	5.42	193.33±5.24	0.21	6.71	1.01
$(NH_4)_2SO_4$	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)*

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)*

Effect of agitation speed

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 at*., 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 (10) : *Effect of agitation on lovastatin production by A. terreus S3γ8 on OPMII.*

Agitation speed (rpm)	Consumed sugar (g/l)	Biomass (g/l)	Lovastatin (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 on the health cares. Lovastatin ($C_{24}H_{36}O_5$) 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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