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# Optimization of process variables for hyper-production of lovastatin from wild type *Aspergillus terreus* and its efficacy studies

# Optimización de variables de proceso para la hiperproducción de lovastatina a partir de *Aspergillus terreus* silvestre y estudios de su eficacia

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**Abstract**

The objective of this study was the investigation of optimum conditions for lovastatin production from *Aspergillus terreus* through response surface methodology (RSM) with central composite design (CCD). The efficacy of produced and purified statin was then evaluated by using induced hypercholesterolemic rats. *A. terreus* was used to synthesize statin by using solid state fermentation on lignocellulose substrate, wheat straw. Total 30 experiments were conducted in triplicate for four physical parameters, each at five levels and response (statin production) from *A. terreus* was measured. Maximum statin formation (60 mg/g) obtained from *A. terreus* at temperature 30 ℃, pH 7.37, inoculum size 4.5 mL and fermentation time 192 h. Hypercholesterolemia was induced in rats by feeding a high cholesterol diet in group I, II and group III from 0 to 30 days. From days 15-30, Group III (treatment group) was given statin extracted from *A. terreus*. Significant reduction in serum cholesterol level in group III rats treated with purified statin were observed at 30$^{th}$ day with significant increase in serum HDL levels along with significant reduction of serum LDL levels.

*Keywords*: Statin, optimization, response surface methodology, *Aspergillus terreus*, lignocellulosic substrate.

**Resumen**

En este trabajo se investigaron las condiciones óptimas para la producción de lovastatina a partir de *Aspergillus terreus* usando una metodología de superficie de respuesta (RSM) con un diseño central compuesto (CCD). La eficacia de la estatina producida y purificada se evaluó en ratas en ratas con hipercolesterolemia inducida. Se empleó *A. terreus* para sintetizar la estatina mediante fermentación en estado sólido usando un sustrato lignocelulósico (paja de trigo). Se realizaron un total de 30 experimentos por triplicado para los cuatro parámetros físicos en cinco niveles y como variable de respuesta se midió la producción de estatina por *A. terreus*. La producción máxima de estatina (60 mg/g) se logró a una temperature de 30 °C, pH 7.37, tamaño de inóculo de 4.5 mL tiempo de fermentación de 192 h. Lla hipercolesterolemia se indujo en las ratas de los grupos I, II and III, alimentándolas con una dieta alta en colesterol durante 30 días. El grupo III (grupo de tratamiento) fue dado la estatina producida por *A. terreus* de los días 15-30, encontrándose que al dí 30 había ocurrido una reducción significativa en el colesterol sérico (Incrementando el significativamente el nivel del colesterol de alta densidad (HDL) y disminuyendo significativamente el colesterol de baja densidad (LDL).

*Palabras clave*: Estatina, optimizacíón, metodología de superficie de respuesta, *Aspergillus terreus*, sustrato lignocelulósico.

## 1 Introduction

The unhealthy life style, lack of exercise and injurious application of cigarette etc. are the major causes of an abnormal blood cholesterol level. CVD (cardiovascular diseases) has a close relation with hypercholesterolemia as it is controlled by combination of lipids and proteins namely low-density lipoprotein (LDL etc.) and high-density lipoprotein (HDL). Appropriate early diagnosis and adequate management using counseling and medication are required for people with CVDs or at high CVD risk (WHO, 2007).

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Atherosclerosis is a multi- factored disease procedure including various well- defined risks factors, like hypertension, diabetes and hypercholesterolemia. About two-third of the blood cholesterol is found in the LDL fraction. Higher LDL cholesterol levels have been favored to increase incidence of coronary artery disease. Natural products also take part a significant role all over the world in curing and safety of human pathogenesis. Naturally formed drugs have come from many natural source materials, like terrestrial vertebrates, terrestrial plants, invertebrates and marine organisms (Dossey, 2010, Bucar *et al*., 2013). The fungal, marine, plant and animal based chemical substances have a deep history of clinical utilization, better patient acceptance and tolerance (Butler, 2008). The first anti-cholesterol drug (Triparnol, 1958) was banned due to its side effect (Kirby 1967) and the second drug ML236B (Compacitin) isolated from *Penicillium citrinum* was also withdrawn owing to its carcinogenic effect (Liu *et al*.,2015).

Finally, the lovastatin (Mevinolin) was discovered from *Aspergillus terreus* which gained popularity due to its multiple therapeutic potential advantages with no side effects (Mukhtar *et al*., 2014; Balraj, *et al*., 2018). SSF (Solid state fermentation) is a biotechnological procedure where microbes, especially fungi, reproduce in insoluble solid substrates at less free water contents (Santos *et al*., 2016; Oliveira *et al*., 2019). Lovastatin was produced as a secondary metabolite of the polyketide pathway by various fungi including *Aspergillus terreus* (Kumar *et al*., 2000). *A. terreus* is known to be the best lovastatin-producing species (Samiee, *et al*., 2003) For instance, (Barrios and Miranda, 2010) stated that solid-state fermentation (SSF) showed better lovastatin yield than submerged fermentation (Smf). Lovastatin production through SSF was optimized by using domestic strain of *A. terreus* (Hassan *et al*., 2019). It can also be manufactured from many fungal strains like. *M. ruber, M. purpereus, T. viridae*, etc. (Nidhiya *et al*., 2012).

In addition, (Suraiya *et al*., 2018) stated that the products obtained can be directly consumed after sterilization. Lovastatin basically most powerful agents existing for decreasing LDL (plasma low density lipoproteins) in cholesterol concentrations (Shitara and Sugiyama, 2006). The best control of lipid pathophysiology in CVD protection could not be attained without the use of statins.

The present work was done to produce maximum lovastatin production through RSM under CCD in solid state fermentation and find out efficacy for purified statin on induced hypercholesterolemic drugs.

## 2 Materials and methods

Whole experimental and analytical work was done in IBL (Industrial Biotechnology Laboratory), Department of Biochemistry, Animal house, CMS Department, University of Agriculture, Faisalabad and Faisalabad Medical University Faisalabad, Pakistan. *Aspergillus terreus* strain was used for statin production. Optimization of physical factors was done through RSM and then purification of whole extract which was applied on animal model. Animals were induced with hypercholesterolemia and the consequence of statin produced under different optimum conditions was investigated.

### *2.1 Fungal strains*

Pure culture of *A. terreus* obtained from IBL, Biochemistry Department, UAF (University of Agriculture Faisalabad). The fungal strain was grown on PDA (potato dextrose agar) slants and spores of *A. terreus* were shifted to the slants by sterile loop in biological hood. Slants were preserved at 4 ºC in refrigerator after incubation for further experiments in statin formation.

### *2.2 Substrate collection and preparation*

Due to inexpensive agro-industrial wastes as substrates SSF is recently take much consideration (Abdullah *et al*., 2018). Wheat straw was dried out in oven at 50 ºC and ground to form a powder (40 mm mesh size). The powder was kept in caped storage jars to maintain free of moisture.

### *2.3 Development of inoculum medium*

Seed cultures of *A. terreus* was grown for 5-8 d in Vogel's medium at pH 6. The medium was autoclaved and sterilized at 121 ºC (Sanyo, Japan) for 15 min. *Aspergillus* spores were then shifted to moderate, cool sterilized inoculum medium within appropriate environment in laminar air flow hood (Dalton, Japan). The inoculated flasks were shaken (150 rpm) at 31 ºC for 5 d (Sanyo-Gallemkemp, UK) to get homogeneous

spore suspension ($1\times10^8$ spores/mL) to be used as inoculum (Kay-Shoemake and Watwood, 1996).

## 2.4 *Optimization of culture conditions by RSM under CCD*

Four physical parameters consisting of pH (A), temperature (B), inoculum size (C) and fermentation time (D) were optimized by RSM using CCD. Different levels of these parameters used in 30 experiments are represented in Table 1.

## 2.5 *Purification of statin*

Crude extracts obtained from *A. terreus* were centrifuged (1000 rpm) for 10 min at 4 ºC and the pellets were discarded. At pH 3.0, adjusted in supernatents using concentrated HCl and extraction was done by mixing of same amount of ethhyl acetate to total sample. In a rotary shaker (180) rpm for 2 h at room temperature, extraction was completed. Centrifugation (1500×g) took place for samples for 15 min. By leaving the aqueous phase, organic phase was collected. The organic part was after than totally volatalized and dry deposit was the purified statin (Goswami *et al*., 2013).

Table 1. Central Composite Design for optimization of statin formation by *A. terreus* in SSF.

| Exp. No. | A: Temp. (ºC) | B: pH | C: In. size (mL) | D:Fermentation time (h) | Response Statin prod. (mg/g) |
|---|---|---|---|---|---|
| 1 | 30 (0) | 6.75 (0) | 4.5 (0) | 192 (0) | 58 |
| 2 | 35 (2) | 8 (2) | 5.5 (2) | 144 (-2) | 39 |
| 3 | 25 (-2) | 5.5 (-2) | 5.5 (2) | 144 (-2) | 40 |
| 4 | 27.5 (-1) | 6.75 (0) | 4.5 (0) | 192 (0) | 57 |
| 5 | 30 (0) | 6.75 (0) | 4.5 (0) | 216 (1) | 55 |
| 6 | 25 (-2) | 8 (2) | 5.5 (2) | 240 (2) | 38 |
| 7 | 32.5 (1) | 6.75 (0) | 4.5 (0) | 192 (0) | 55 |
| 8 | 30 (0) | 6.75 (0) | 5 (1) | 192 (0) | 58 |
| 9 | 35 (2) | 8 (2) | 3.5 (-2) | 144 (-2) | 40 |
| 10 | 25 (-2) | 5.5 (-2) | 5.5 (2) | 240 (2) | 45 |
| 11 | 30 (0) | 6.75 (0) | 4.5 (0) | 192 (0) | 55 |
| 12 | 30 (0) | 6.75 (0) | 4.5 (0) | 192 (0) | 59 |
| 13 | 30 (0) | 6.75 (0) | 4.5 (0) | 192 (0) | 57 |
| 14 | 30 (0) | 6.75 (0) | 4.5 (0) | 192 (0) | 58 |
| 15 | 30 (0) | 6.125 (-1) | 4.5 (0) | 192 (0) | 57 |
| 16 | 30 (0) | 6.75 (0) | 4.5 (0) | 192 (0) | 58 |
| 17 | 35 (2) | 5.5 (-2) | 3.5 (-2) | 240 (2) | 36 |
| 18 | 35 (2) | 5.5 (-2) | 5.5 (2) | 240 (2) | 40 |
| 19 | 35 (2) | 5.5 (-2) | 5.5 (2) | 144 (-2) | 43 |
| 20 | 25 (-2) | 8 (2) | 5.5 (2) | 144 (-2) | 38 |
| 21 | 35 (2) | 8 (2) | 3.5 (-2) | 240 (2) | 39 |
| 22 | 25 (-2) | 5.5 (-2) | 3.5 (-2) | 144 (-2) | 39 |
| 23 | 30 (0) | 6.75 (0) | 4 (-1) | 192 (0) | 56 |
| 24 | 30 (0) | 7.375 (1) | 4.5 (0) | 192 (0) | 60 |
| 25 | 35 (2) | 8 (2) | 5.5 (2) | 240 (2) | 37 |
| 26 | 25 (-2) | 8 (2) | 3.5 (-2) | 144 (-2) | 40 |
| 27 | 35 (2) | 5.5 (-2) | 3.5 (-2) | 144 (-2) | 40 |
| 28 | 25 (-2) | 8 (2) | 3.5 (-2) | 240 (2) | 42 |
| 29 | 30 (0) | 6.75 (0) | 4.5 (0) | 168 (-1) | 55 |
| 30 | 25 (-2) | 5.5 (-2) | 3.5 (-2) | 240 (2) | 45 |

*Coded values are given in bracts after actual levels of parameters.

### *2.6 Biological evaluation of statin in rats*

Hypocholesterolemic effect of statin extracted from fungi was investigated in hypercholesterolemic rats. Healthy male white rats (albino, Wistar strain) weighed about 150 - 200 g were bred in CMS (Clinical, Medicine and Surgery) Department (animal house), Univerisity of Agriculture, Faisalabad for hypocholesterolemic action of statin extracts from *A. terreus* strains. The study was carried out according to internationally accepted principles for labortory animal use and care as per the U.S. guidelines (NIH publication No. 85-23, revised in 1985) using 20 rats.

### *2.7 Materials used for effficacy studies*

Following materials, in different dose regimens and rations, were used for efficay studies of statins against induced hypersholesemia:

i Normal rodent diet g /100 g (flour (82), casein (4.0), minerals (1.0), vitamins (3.0) and corn oil 10 mL used as standard diet for control subjects.

ii Cholesterol rich diet g /100 g ( flour (80.5), cholesterol (1.5), casein (4.0), minerals (1.0), vitamines (3.0) and corn oil (10 mL) used to induce

iii Purified extract of statin obtained from A.terreus induced SSF based lignocellulosic biomass

### *2.8 Operational trial*

Whole rat groups were weighed and distributed haphazardly among three groups. Group I (normal control gp), Group II (Cholesterol control gp) & Group III (Treatment gp ) at day 0. Each group carried five animals and all the groups were kept in standard situation for 30 d. Group I had given normal standard diet while animals in group II & III were offered the rich cholesterol diet respectively.

### *2.9 Biochemical parameters*

After treatment with statin on group III, total cholesterol (TC), HDL-C, triglycerides and LDL-cholesterol were measured in the rat sera by adopting the protocol outlined in the manufacturer's assay kit (Human) on all sera of whole groups. From orbital sinus of rats, blood samples were taken by capillary tube. Biochemical analysis were done at different days (0, 15, 30) for whole animals in all distributed groups. The rat sera were preserved in deepfreezer and investigated with in 3 d. LDL-C was estimated using the Friedewald formula −LDL−C=TC− [HDL-C + TG/5). Estimation was made using Auto analyzer (ERMA Inc.).

### *2.10 Analysis of lovastatin*

For UV spectrophotometric analysis, 1 mL of clear supernatant was mixed with 1mL of tri floro acetic acid and the incubation was given to mixture for 10 minutes for lactonization of hydroxyl acid form of statin (Ragunath *et al*., 2012). The resulting mixture was mixed with methanol and the absorbance was read at 238nm using UV-VIS Double Beam spectrophotometer (Dynamica). Standard curve of statin was constructed by plotting concenterations verses absorbances. Pure lovastatin lactone (99.9%) form (Sigma Aldrich) was dissolved with methanol to make pure mixture of varying concenterations (1.0 mg-10.0 mg/mL) methanol in test tubes and make volume up to 1.0 mL with methanol and read their absorbances at 238 nm.

### *2.11 Statistical Analysis*

Two-way factorial design using Statistix version 10 was used for the optimization of response. Statistically significant was measured through $p$-value less than 0.05.

## 3 Results and discussion

The current work was put away to investigate the potency of *A. terreus* for statin formation by cultivating it on wheat straw. Four factors each of five levels in Central Composite Design needed 30 runs in triplicate executed in this study. Response (statin formation) from *A. terreus* was observed. Experimental designs including RSM are a statistical technique to evaluate optimization of independent changeable values, so as to attain best yield and make the user to evaluate the interaction of single changeable value, considering more effective than the usual single variable for best conditions due to saving of raw material, space and time (Felix *et al*., 2018). It is an assembly of mathematical techniques and statistics and helpful for developing, improving and optimizing fermentation procedure. The significance of RSM is the less experimental runs required to investigate numerous factors at a time and their

interactions (Karacan *et al*., 2007). Utilization of RSM has built up optimum concentration for researchers and process factors (Dey *et al*., 2002).

The data obtained from various runs employed were investigated and depicted by using design of expert (DOE) version 6.0.8. CCD describes nature of response surface in best place (Pratheebaa *et al*., 2013). Statistical based optimum conditions not only permit rapid testing of a big study place, but shows the position of every unit too. Best statin formation obtained from A. terreus at temperature 30 ºC, pH 7.37, inoculum size 4.5 mL and fermentation time 192 h was 60 mg/g Table 1.

## *3.1 Regression analysis*

The usage of statistical experimental design techniques in fermentation procedure progress development and can affect in improving product formation, decrease procedure diversity, give about real specification of the outcome to nominal and less whole expenditure. It is a speculative statistical modeling procedure also used for multiple regression investigation using accessible data. Response surface methodology is common to investigate the interactions among various parameters and the response surface (Baseman *et al*., 2013). The outcomes available from Response Surface Methodology (RSM) managed to set a second order polynomial equation for clarifying about performance of the system among cross product and quadratic expression.

$$Y = \beta_0 + \sum \beta_i X_i + \sum_{ii} X_i^2 + \sum \beta_{ij} X_i X_{ji} \qquad (1)$$

where $Y$ is the response variable (statin formation). The complete multiple regression equation analysis showing the subsequent regression equation for statin formation by *A. terreus*.

$$\begin{aligned} Y2 = {} & (57.62) + (-0.85A) + (-0.82B) + (0.000C) \\ & + (0.18D) + (-6.89A^2) + (3.11B^2) + (-2.89C^2) \\ & + (-10.89D^2) + (.44AB) + (0.56AC) + (-1.44AD) \\ & + (-1.06BC) + (-0.31BD) + (-0.19CD) \end{aligned} \qquad (2)$$

Through analysis of variance (ANOVA), proposed model adequacy was exposed by using the diagnostic checking tests (Abdullah *et al*., 2018). Coefficient of determination $R^2$ was frequently utilized to investigate the suitability association with in predicted model and observed records. The value of $R^2$ was between 0 and 1. The closer the $R^2$ value to 1, the better the fitness of the model to observed data (Ruchir *et al*., 2010). $R^2$ mainly investigate the variation percent (Chen *et al*., 2009, Pratheebaa *et al*., 2013).

Predicted $R^2$ 0.9259 for *A. terreus* by the models (statin production) were in near accordance with adjusted $R^2$ i.e. 0.9726 *A. terreus*. Adjusted $R^2$ to actual $R^2$ value in statin formation by *A. terreus* showed that the linear, square and interaction terms could describe 98.58% of variation with the illustrate of satisfactory expression the method of model. The accuracy along with dependability in operated runs proved through less means of coefficient of variation of 3.06% for *A. terreus*. "Adeq Precision" measures the signal to noise ratio. A ratio larger than 4 is wanted. In our case all model ratios 21.436 (*A. terreus*) were larger than prescribed adequate signals. These employ navigated the pattren space. The values of standard deviation exposed that model had powerful similarity to the proposed outcome.

The Model F-value of 74.479 implies the model is significant (Table 2). Only 0.01% chance is there due to this large value could occur due to noise. In this case A, B, $D^2$, AD, BC were significant terms. Lack of fit was non-significant that confirms the fitness of model.

## *3.2 Correlation analysis*

A close correlation can be seen in actual and predicted values for statin formation by *A. terreus* in SSF (Fig. 1). A powerful correlation within real and supposed values used to investigate accuracy of CCD (Zhu *et al*., 2011).

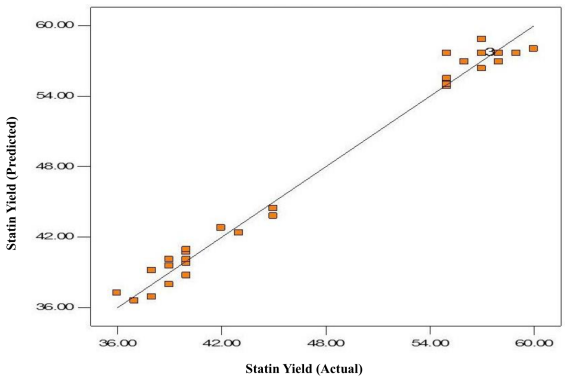


Fig. 1. Correlation of statin levels were plotted for actual values obtained after fermentation by *A. terreus* in SSF and analyzed versus predicted values obtained by CCD.

Table 2. ANOVA table for (quadratic polynomial model) for statin production by *A. terreus*.

| Source | Sum of Squares | DF | Mean Square | F Value | *p* |
|---|---|---|---|---|---|
| **Model** | **2242.704** | **14** | **160.1931** | **74.47918** | **< 0.0001** |
| **A** | **11.87879** | **1** | **11.87879** | **5.522848** | **0.0329** |
| **B** | **11.04545** | **1** | **11.04545** | **5.135403** | **0.0387** |
| C | 0 | 1 | 0 | 0 | 1 |
| D | 0.545455 | 1 | 0.545455 | 0.2536 | 0.6219 |
| A2 | 7.899012 | 1 | 7.899012 | 3.672516 | 0.0746 |
| B2 | 1.610137 | 1 | 1.610137 | 0.748607 | 0.4005 |
| C2 | 1.389443 | 1 | 1.389443 | 0.645999 | 0.4341 |
| **D2** | **19.73394** | **1** | **19.73394** | **9.174972** | **0.0085** |
| AB | 3.0625 | 1 | 3.0625 | 1.423859 | 0.2513 |
| AC | 5.0625 | 1 | 5.0625 | 2.353726 | 0.1458 |
| **AD** | **33.0625** | **1** | **33.0625** | **15.37187** | **0.0014** |
| **BC** | **18.0625** | **1** | **18.0625** | **8.397863** | **0.011** |
| BD | 1.5625 | 1 | 1.5625 | 0.726459 | 0.4074 |
| CD | 0.5625 | 1 | 0.5625 | 0.261525 | 0.6165 |
| Residual | 32.26267 | 15 | 2.150845 | | |
| Lack of Fit | 22.76267 | 10 | 2.276267 | 1.198035 | 0.4461 |
| Pure Error | 9.5 | 5 | 1.9 | | |
| Total | 2274.967 | 29 | | | |

Significant values are represented by **bold** letters/numbers.

Bizukojc *et al*., (2012) reported that neutral and basic pH range produced greater lovastatin formation as acidic ones This is due to effect for polyketide pathways (*A. terreus*) and the substantial reduce of (+) geodin (Bizukojc and Ledakowicz, 2008). pH also influences lovastatin production (Bizukojc *et al*., 2012) as pH powerfully effects the transportation of many ingredients through the physiological membrane (Mouafi *et al*., 2016).

Inoculum size in SSF has been studied positive and negative influences. Various inoculum sizes when greater or lesser (Jahromi *et al*., 2012). However, may greater or lesser effect on lovastatin formation (Ruchir *et al*., 2010, Jahromi *et al*., 2012). Another factor temperature was significant physical parameter to progress lovastatin production by accelerating genes or enzyme actions (Pansuriya and Singhal, 2010). The greater lovastatin production related SSF was to enhance mycelia production (Li *et al*., 2005, Seenivasan *et al*., 2008) and greater penetrability (Reddy *et al*., 2011)). A study reported on *Omphalotus olearius* by Atli *et al*., (2016) gave 2.23 mg/g (lovastatin) under non-optimal circumstances. While under best situations, experimental value was 139.47 mg/g (lovastatin) substrate.

### *3.3 Interaction between variables*

The interaction among independent variables and dependent variable (statin production) in case of *A. terreus* can be analyzed graphically by 3 D response surface plots that can utilized to estimate best situations. The interaction among inoculum size and temperature was significant and it effected statin formation. Inoculum size was 4.5 at temperature 30 ºC for maximum yield of statin in *A. terreus* (Fig. 2).

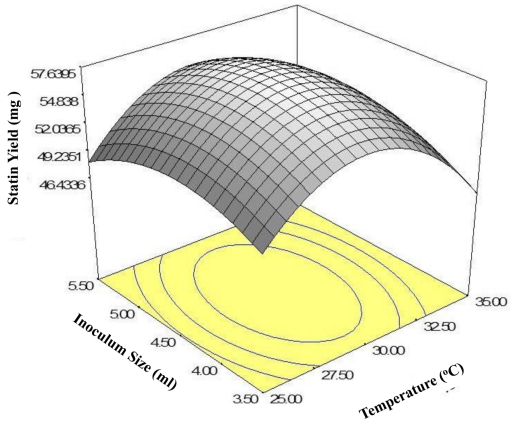


Fig. 2. The interaction between inoculum size and temperature. A significant interaction was observed for satatin production, 4.5 ml of *A . terreus* inoculum yielded optimum level of statin at 30 ºC.

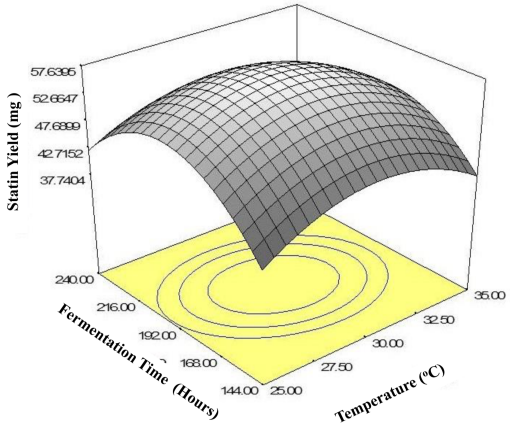


Fig. 3. The interaction between fermentation time and temperature. A significant interaction was observed for satatin production, *A. terreus* yielded optimum level of statin after 192 hours at 30 ºC.

The interaction between fermentation time and temperature was was also significant and has effect on statin formation (Fig. 3). Best yield was achieved at 30 ºC temperature and 192 h fermentation time for *A. terreus*.

### *3.4 Biological effect of statin formed on animal model*

Hypercholesterolemic animals are beneficial models for observing the cholesterol management and drug experiments to link the abnormalities in cholesterol homeostasis, thermogenesis along with potential actions for reducing plasma cholesterol amount (Jung and Wang, 2009). High cholesterol level was induced in rats (Wistar) specie by feeding a high cholesterol diet. Purified extract was given to group III only from day 15 till day 30. All the experimental animals were in better health conditions during the whole trial. Average body weights of the animals were also measured each week during the experiment. Carboxy methyl cellulose (CMC) was utilized (0.25% w/v) as vehicle for purified statin extracts from *A. terreus* through rat feeding tube.

### *3.5 Blood cholesterol*

The Blood cholesterol for all groups were measured at day first (day 0) as baseline values. Repeat investigations of serum cholesterol level were estimated on days 15 and 30 too (Table 3). Group I (control rats) at day 15 had a mean±SEM blood cholesterol level of 61±4.3mg%. In group II (the cholesterol control group), the value was 77.2±2.6 mg% on day 15 showing a notable increase from the baseline value. There was significant fall in blood cholesterol level on day 30 (group III) treated with purified statin extract from *A. terreus* source.

Two -way factorial ANOVA table showed that there was significant decrease in cholesterol level when purified statin extracts from *A. terreus* was given to group III ($p > 0.05$). This group was induced hypercholesterolemia.

Table 3. Serum cholesterol level analyzed at 0 day, 15$^{th}$ day and 30$^{th}$ day.

| Treatments | day 0 | day 15 | day 30 | Mean |
|---|---|---|---|---|
| **GpI** | 62.2±3.9 | 61±4.3 | 60.6±1.3 | $61.3\pm3.2^{a}$ |
| **GpII** | 64.2±1.3 | 77.2±2.6 | 90.4±3.6 | $77.3\pm2.5^{a}$ |
| **Gp III** | 64.4±3.2 | 86.2±2.8 | 64.4±3.4 | $71.7\pm3.1^{a}$ |
| **Mean** | $61.9\pm2.7^{a}$ | $77.95\pm3.4^{a}$ | $70.6\pm3^{a}$ | |

Table 4. Serum triacylglyceride level analyzed at 0 day, 15$^{th}$ day and 30$^{th}$ day.

| Treatment | 0day | 15day | 30day | Mean | % fall in TGs level |
|---|---|---|---|---|---|
| **GpI** | 86.8±6.3 | 101.4±4.2 | 160.8±8.8 | $116.3\pm6.4^{b}$ | -35.3±13.8 |
| **GpII** | 89.8±3.8 | 110.2±3.6 | 164.6±8.8 | $121.5\pm5.4^{ab}$ | -49.4±8.5 |
| **GpIII** | 89.4±4.6 | 106.4±6.1 | 170±5.4 | $121.9\pm5.4^{ab}$ | -60.2±11.1 |
| | $88.5\pm5.0^{c}$ | $108.9\pm5.7^{b}$ | $166.65\pm6.9^{a}$ | | |

*Negative values indicate increase in triglyceride levels

Table 5. Serum HDL levels in induced hypercholesterolemic rats after giving statin extracts (*P. spodoecus, A. terreus*).

| Treatment | 0 day | 15 day | 30 day | Mean | % fall in HDL level |
|---|---|---|---|---|---|
| **GpI** | 21.8±2.4 | 25.4±3.8 | 23±4.7 | 23.4±3.6[a] | 8.7±17.9 |
| **GpII** | 26.4±6.1 | 18.6±3.0 | 11.6±2.1 | 18.9±3.7 a | 36.88±11.1 |
| **GIII** | 25.6±5.2 | 13.2±4.5 | 19.2±6.6 | 19.3±5.4 a | -33.46±40.4 |
| | 26±4.3[a] | 18.1±3.6[a] | 18.8±4.5[a] | | |

*Negative values indicate increase in serum HDL levels.

### *3.6 Serum triacylglycerol (TG) level*

Serum triglycerides level was also investigated at days 0, 15$^{th}$ and 30$^{th}$. On day 30$^{th}$ control group (group I) had a mean ± SEM serum TG value was 160.8±8.8 mg%. In group II (cholesterol control group) the level was 164.6 ± 8.8 mg%. While group III had 170±5.4 mg% on day 30$^{th}$ respectively, as represented in Table 4.

### *3.7 Serum HDL level*

At day 30 Mean ± SEM serum HDL value of group I (normal control group) had 23±4.7 mg%. In group II (the cholesterol control rats) the level was 11.6±2.1mg%. Group III treated with purified extracts from *A. terreus*, serum HDL value was 19.2± 6.6 mg% (Table 5).

Diet (oyster mushroom) with 10% dry fruiting bodies especially lowers the presence and size of atherosclerotic plaques in rabbits. Lovastatin with the HMG-Co AR was recognized in this oyster mushroom and might be the chief part susceptive for the investigative impacts (Jung and Wang, 2009).

The devastating indication of both vascular protective and cardioprotective benefits of statin therapy and the constant detection of activities, these inhibitors revealed that the management of blood pressure through statins may take part in the hypertensive and hyper lipidemic- patient (Chopra *et al*., 2007). A combined effect of statins showed capacity of decreasing LDL-C and BP. (Das *et al*., 2008, Schneider *et al*., 2008) studied that diet containing boiled soup with oyster showed notable effect on human lipid profile. These observations were accordance to our observations in which serum TC level was significantly reduced and also according to number of animal trials in which significantly reduced TC levels in involvement of oyster mushrooms (Hossain *et al*., 2011). Managing LDL-C is favored on total cholesterol because it linked to risk factors (McBride, 2008).

## Conclusions

Domestic strain of *A. terreus* was found to be a very potent strain capable to produce statin in solid state fermentation settings. Wheat straw was proved to a suitable lignocellulose substrate for statin production. All experiments were conducted in triplicate for four physical parameters, each at five levels and response (statin production) from *A. terreus* was measured. A very reasonable amount of statin (60mg/g) was obtained from A. terreus at temperature 30 ºC, pH 7.37, Inoculum size 4.5 mL and fermentation time 192 h. Hypercholesterolemic rats fed with high cholesterol diet in group II and group III from 0 to 30 days were investigated for controlling hypercholestemia using statin produced form *A. terreus* through SSF and significant reduction in serum cholesterol level in group III rats, treated with purified statin, were observed at 30$^{th}$ day with significant increase in serum HDL levels. Additionally, significant reduction of serum LDL levels was also observed. This clearly showed that produced statin was of good quality and no toxic effects were developed in rats and well controlled cholesterol level in rats.

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