# Optimization of process variables for increased production of lovastatin in *Aspergillus terreus PU-PCSIR1* and its characterization

**Abida Hassan[1,2], Yasar Saleem[1]*, Muhammad Nawaz Chaudhry[2], Amina Asghar[4], Muhammad Saleem[3], Shaista Nawaz[1], Quratulain Syed[1], Muhammad Shaheen Iqbal[1] and Khurram Shahzad[1]**

[1]Food and Biotechnology Research Centre, PCSIR Labs Complex, Ferozepur Road, Lahore, Pakistan
[2]College of Earth and Environmental Sciences, University of the Punjab, Lahore, Pakistan
[3]Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[4]Division of Science and Technology, University of Education, Lahore, Pakistan

**Abstract**: During intrinsic cholesterol formation 3-hydroxy-3-methylgutaryl coenzyme A reductase (HMGCR) converts HMGCoA to mevalonate, in biosynthetic cascade of cholesterol. Statins, competitive inhibitors of HMGCR, now-a-days commonly used to lower the blood-cholesterol level in the hyper-cholesterolemic patients. Lovastatin, one of the most potent natural statins, was produced from wild-type indigenous isolate *Aspergillus terreus* PU-PCSIR-1, through solid state fermentation (SSF). This study was carried out to investigate different parameters influencing lovastatin production such as pH, carbon source, nitrogen source and media components etc. Each parameter was investigated separately to optimize lovastatin production. Maximum yield of 2860mg/Kg of total lovastatin, comprising 1700 and 1160mg/Kg of hydroxy and lactone forms respectively, was achieved after incubating for 14 days, pH 5.5 and at 28°C. The integrity of biotechnologically-produced lovastatin was analyzed using high performance liquid chromatography (HPLC). Lovastatin was purified by preparative HPLC, and was characterized by FT-IR and LC-MS analyses. The study revealed that *A. terreus* PU-PCSIR-1 has been proved to be a potent strain for the production of lovastatin that has great pharmaceutical and commercial applications.

**Keywords**: *Aspergillus terreus*, high performance liquid chromatography (HPLC), Lovastatin, submerged fermentation.

## INTRODUCTION

Lovastatin ($C_{24}H_{36}O_5$, mevinolin, monacolin K), a member of hypocholestroleimic group of drugs (statins), reduces human plasma cholesterol level in blood (Alberts *et al*., 1980; Tobert, 1987 and Barrios-Gonzalez and Miranda, 2010). More than 50 % of cholesterol synthesis in human body is attributed to de-novo synthesis (Grundy, 1978), which follows complex pathway comprising contribution of more than thirty enzymes (Bergstrom, 1984 and Tobert, 2003). Among these enzymes a microsomal enzyme, 3-hydroxy-3-methyl glutaryl Co-enzyme A Reductase (HMGCR), converts HMGCoA to mevalonate (Rodwell *et al*., 1976; Springer, 1980 and Brown, 1980), a rate-limiting step in cholesterol biosynthetic pathway (Alberts *et al*., 1980 and Tobert, 1987). Lovastatin halts this rate limiting step by inhibiting HMGCR which results in decreased level of mevalonate (Balasubramaniam, 1977 and Pappu and Illinworth, 1989), thereby controlling cholesterol biosynthesis. The first natural HMGCR inhibitor, compactin, was isolated from fermented broth of *Pencillium citrinum* (Endo *et al.*, 1976 and Endo, 1979). However, a more potent HMGCR inhibitor, mevinolin later renamed as lovastatin, was discovered in fermentation broth of *Aspergillus terreus*. It was launched in pharmaceutical market as US - FDA approved cholesterol reducing drug which, soon became one of the most successful hypocholestrolemic agents (Tobert, 2003). In addition to predominant efficacious results in cholesterol reduction and cardiovascular therapy statins have important modulatory effects on oxidative stress, endothelial function, coagulation, inflammation (Khanicheh *et al*., 2013) and plaque stability.

Lovastatin, as a secondary metabolite was produced by numerous fungal species including Monascus *(M) purpureus, M. ruber, M. pilosus, M. pubigerus M. vitreus* (Endo, 1979; Negishi *et al*., 1986 and Manzoni, 1998) and *Aspergillus terreus* (Alberts *et al*., 1980; Novak, 1997; Szakacs, 1998; Rodriguez, 2007; Bizukojc and Ledakowics, 2007 and Jia *et al.*, 2010) using submerged (SmF) fermentation and solid state fermentation (SSF) processes (Barrios-Gonzalez, 1996 and Molina and Chisti, 2003). Many researchers compared SSF and SmF and found SSF a better process due to the higher yield of lovastatin production (Robinson *et al*., 2001; Balakrishna and Pandey, 1996; Holker and Lenz, 2005; Barrios-Gonzalez and Mejia, 2007 and Thomas *et al*., 2013). Initially, lovastatin was produced, at industrial scale, by SmF but later SSF became the method of choice (Suryanarayan, 2003).

Present study was carried out to optimize lovastatin yield by examining the influence of different process variables

**Corresponding author:* e-mail: ysaleem73@hotmail.com

on lovastatin production by using indigenous strain *Aspergillus terreus* PU-PCSIR1 through SSF. For this purpose single factor multiple level and multiple factor single level experiments were designed to enhance lovastatin yield by using wheat-bran as solid substrate. Wheat-bran was selected due to its economic availability. Various parameters including initial pH of the medium and different nutritional factors like concentrations of peptone, lovastatin production media (LPM), glycerol and soybean powder were optimized to maximize lovastatin yield. Furthermore purity and integrity of product was confirmed by reverse-phase high performance liquid chromatography (RP-HPLC), Fourier-Transform Infrared (FT-IR) Spectroscopy, and Liquid Chromatography Mass Spectroscopy (LC-MS).

## MATERIALS AND METHODS

***Strain isolation and sub-culturing***

Fungal strain *Aspergillus terreus* PU-PCSIR 1 was a local isolate from soil of College of Earth and Environmental Sciences (CEES) and identified by plant pathologists at Institute of Agricultural Sciences, University of the Punjab, Lahore. The fungal strain was grown onto potato dextrose agar (PDA) at 28$^{o}$C for seven days until complete sporulation. The slants were maintained at 4$^{o}$C and were sub-cultured periodically for further use.

***Preparation of inoculum of Asperegillus terreus***

*A. terreus* was cultured in growth and production phases. In growth phase, spore suspension was obtained by washing the slant culture with 10ml sterile aqueous-solution containing 0.9% NaCl and 0.05% Tween-20. The spores were then transferred into 250ml Erlenmeyer flask having 100ml of inoculum medium containing: 10g glucose, 5g corn steep liquor, 40g tomato paste, 10 g oat flour, and 10 ml trace elements solution per liter. The trace elements solution, a component of inoculum medium, composed of: 1g of $MnSO_4.4H_2O$, 1g of $FeSO_4.7H_2O$, 200mg of $ZnSO_4.7H_2O$, 56 mg $H_3BO_4$, 100mg of $CaCl_2.2H_2O$ and 19mg $(NH_4)_6\ Mo_7O_{24}.4H_2O$ per liter of the solution (Alberts *et al*., 1980), pH was adjusted to 6.8 before sterilization. Inoculum medium was then incubated at 28$^{o}$C for 24 hours on a rotary shaker, adjusted at 220rpm. After 24 hours of incubation, the spore suspension was aseptically filtered. Spore count was maintained at $10^6$ spores/ml and an amount of 5ml of spore suspension was used as inoculum. In production phase, conditions for lovastatin production were optimized using SSF.

***Lovastatin production by solid-state fermentation (S.S.F.)***

Solid-state fermentation was carried out in 250ml Erlenmeyer-flasks containing 30g$^{-L}$ wheat bran. The moisture level and pH of lovastatin production media (LPM) was maintained at 80 % and 6.8 respectively. LPM contained: 25g glucose, 50g lactose, 2.5g yeast-extract, 24g peptonized-milk, 1.25g mono sodium glutamate, 10ml trace element solution and 30µL of linoleic acid per liter. The flasks containing LPM were autoclaved; spores were transferred aseptically and incubated at 28$^{o}$C for 14 days. All the experiments were performed in triplicate and average values were presented. Effect of different process variables including pH, carbon source, nitrogen source etc was studied on lovastatin yield.

***Extraction of lovastatin from fermented biomass***

After 14 days of static SSF, the fermented material was dried at 60$^{o}$C for 24 hours and was ground into fine powder under sterile conditions. Lovastatin was then extracted from solid culture media by shaking 3.0g of solid biomass with 30ml of methanol on a rotary shaker (180rpm) for 2 hours. In order to rupture the fungal mycelia and isolate lovastatin, biomass was sonicated by probe sonicator (Ultrasonic generator, Model US-300). Extract was filtered and concentrated by evaporating the solvent at ambient temperature. The samples were then subjected to analysis on RP-HPLC.

***Quantitative analyses of lovastatin by RP-HPLC***

Concentrated lovastatin samples were filtered through syringe filters (Millipore, 0.22µm) and quantified by HPLC (Perkin Elmer, Series 200), studded with quaternary pump, on-line degassing system, reverse phase $C_{18}$ column (Shim-Pack CLC-ODS (M), 6mmx150mm, particle size 5µm) accompanied by CLC-ODS guard column and Perkin Elmer, Series 200 auto sampler. The chromatographic conditions were: Mobile phase comprising acetonitrile and 0.1% aqueous phosphoric acid (60: 40), flow rate 1ml/min, injection volume 10µl. Detection was performed at 238nm using Perkin Elmer UV/Vis. detector (Series 200). *β*-Hydroxyl form of lovastatin was prepared from lactone form by using the method of Friedrich *et al* (Friedrich *et al*., 1995). Lovastatin was quantified as lactone and *β*-hydroxyl forms by comparing with lovastatin lactone standard (Sigma). TotalChrome 3.2.2 software was used to operate instrument, data acquisition and data analysis.

***FT-IR analysis***

Confirmation of lovastatin from extracted samples was done by FT-IR (Thermo Nicolef 200, USA) analysis. Purified lovastatin was crystallized by keeping the sample in refrigerator for two weeks. Small amount of crystallized sample was prepared in KBr and scanned for absorbance over a range from 4000 to 500 wave numbers ($cm^{-1}$) and spectra were recorded.

***LC-MS analysis of lovastatin***

Mass spectra of extracted lovastatin was measured on 6224 TOF LC/MS (Agilent Technologies, USA) equipped with dual electrospray ionization source. The data were

acquired using Mass Hunter Workstation software for quantitative analysis (version B.01.02) drying gas flow rate 5ul/min, drying gas temperature 300°C, fragmentor voltage was 175 V. Samples were injected on reverse phase RP-HPLC column (Poroshell 300SB-C-18: length 75mm, internal dia 2.1mm particle size 3µm, Agilent Tech., USA). Separation was done using with mobile phase: A - 0.1% formic acid, B - acetonitrile+0.1% formic acid, using gradient of 15µl/min: 5min - 60%, 15-20min - 90%, 25min - 60% of mobile phase B.

## RESULTS

### *Effect of pH on lovastatin production*

During this single factor multiple level experiments initial pH of the samples varied from 5.0 to 8.0. It was observed that highest lovastatin yield was achieved at pH5.5. Further increase in pH reduced lovastatin yield (fig. 1). The activities of the secondary metabolites during fermentation depends upon pH, and a change in pH might alter them (Kysilka, 1993) possibly by changing the permeability of cell membrane by metallic ions, which resulted in enhanced lovastatin production (Madan and Thind 1998). Based on previous reports selected pH variation was carried out. According to some reports pH range of 5-7 is suitable for optimum production of statins from fungi (Panda *et al*., 2010, Shaligram *et al.*, 2008). Jahromi *et al.*, 2012 stated that pH less than 6 and more than 7 affects negatively on the lovastatin production. However, Manzoni and Rollini 2002 described five times increase in lovastatin production under controlled pH and slow addition of carbon source. Panda *et al.*, 2009 attained optimum yield of lovastatin by solid state fermentation of *M. purpureus* at pH 6 whereas Prabhakar *et al.*, 2012 achieved maximum yield of lovastatin by mutant strain of *A. terreus* at a pH 5.5. Therefore pH range of 5-7.5 was selected for optimum yield of lovastatin.

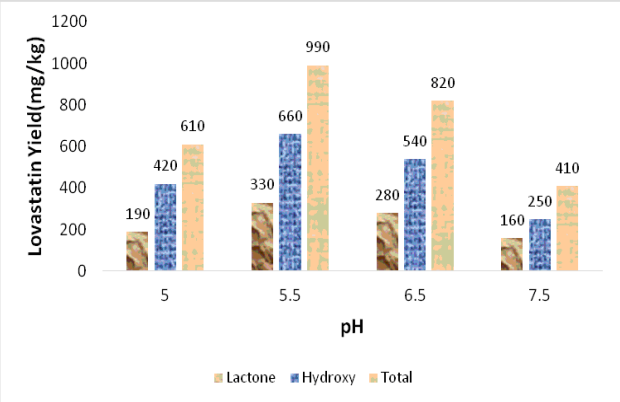



**Fig. 1**: Effect of pH (5.0-7.5) on lovastatin production by *Aspergillus terreus* in SSF. Every bar indicates the mean yield of three replicates.

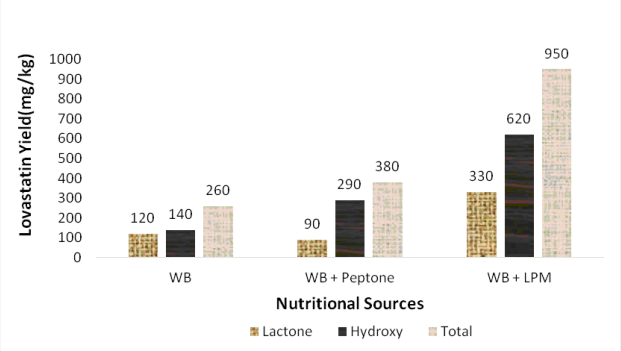



**Fig. 2**: Effect of various media components on lovastatin yield by *Aspergillus terreus* in SSF. Wheat bran only as control (WB) was supplemented with nutritional components including Peptone (WB + Peptone), Glucose and complex media LPM (WB+LPM). Both lactone and β-hydroxy forms of lovastatin were produced and yield was increased approximately 3 times by the addition of additional nutritional supplements. WB and LPM in figure represents wheat bran and lovastatin production medium.

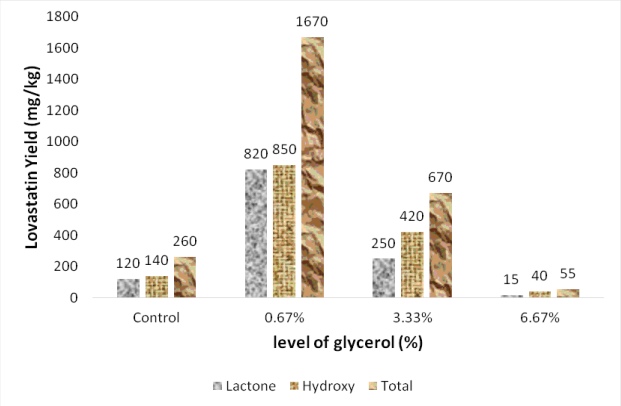



**Fig. 3**: Effect of glycerol (0.67% to 6.67%), an additional carbon source, on lovastatin yield by *A.terreus* in SSF. Both lactone and β-hydroxy forms of lovastatin were produced and yield was increased significantly on adding 0.67% glycerol.

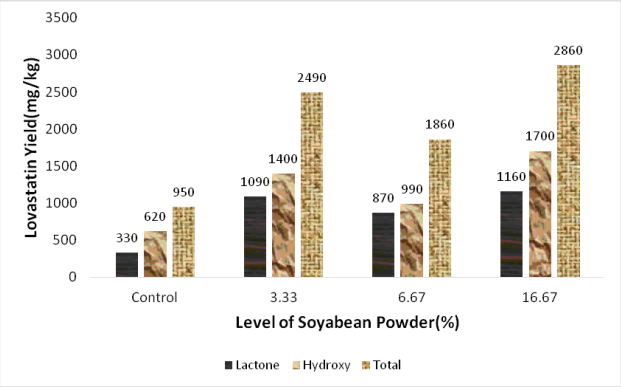



**Fig. 4**: Effect of complex nitrogen source, soybean powder, on lovastatin yield. Different concentrations of

soybean powder from 3.33% to 16.66% were supplemented to control experiment and an approximately 3 fold increase in total lovastatin yield was observed.

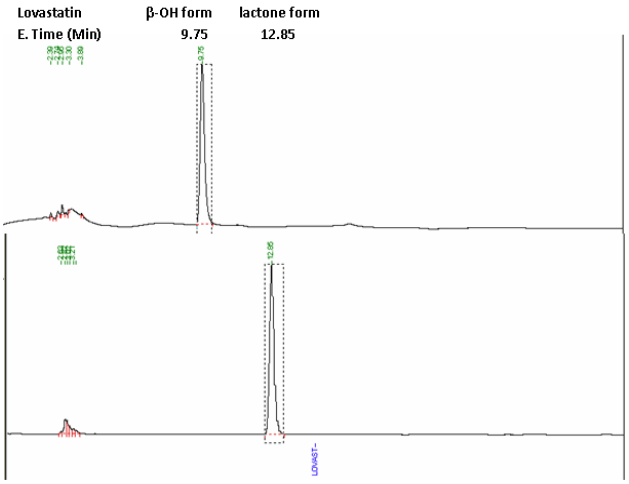
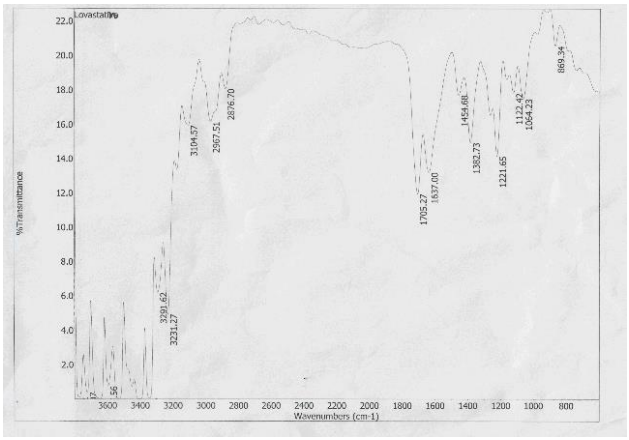



**Fig. 5**: HPLC analysis of different forms of lovastatin. Chromatogram of *β*-hydroxy form of lovastatin (HL) (upper panel); and lactone form of lovastatin (LL) (lower panel) analyzed by RP-HPLC using $C_{18}$ column.

A




B

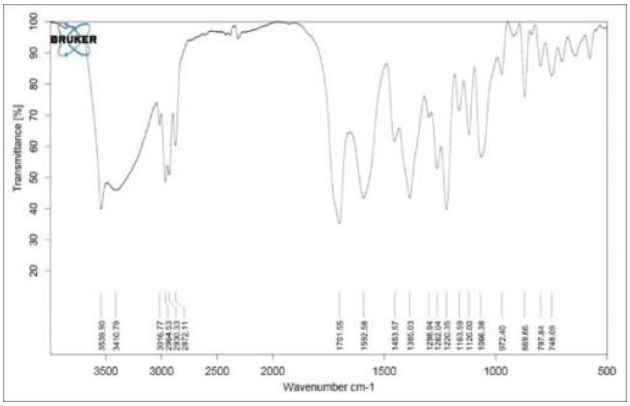



**Fig. 6**: FT-IR spectrum of biosynthesized lovastatin from *Aspergillus terreus* PU-PCSIR1 (A). FT-IR spectrum of standard lovastatin, adapted from Bruker Inc. (www.bruker.com) catalogue (B).

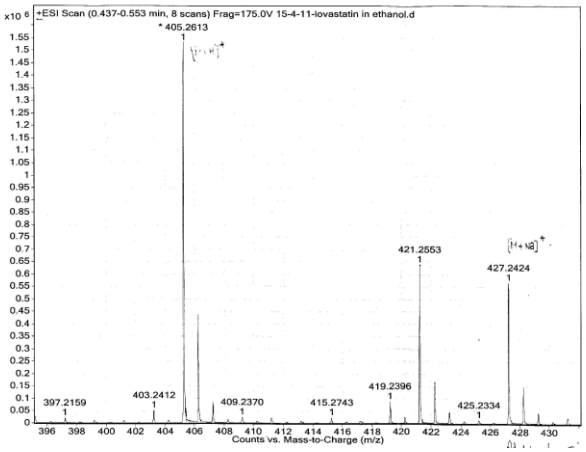



**Fig. 7**: ESI-MS (+ve) analysis of lovastatin. Peak at *m/z* 405.2613 represents lovastatin lactone form while peak at *m/z* 421.2553 represents beta hydroxyl acid form of lovastatin. Na-adduct ion of lovastatin appeared at *m/z* 427.2427.

***Effect of different nutritional sources on lovastatin production***

In this experiment wheat bran, supplemented with glucose, was used as basal control medium for lovastatin production. As compared to control, lovastatin yield was significantly enhanced by the addition of different nutritional components such as peptone and LPM. It was noticed that lovastatin yield increased from 260mg/kg to 950mg/kg. This rise was attributed to the addition of LPM, having combination of different simple and complex nutrients. Total yield of lovastatin was approximately 3 times higher in LPM supplemented with wheat bran than basal control medium (fig. 2). Combination of rapidly metabolizing sugars and slow metabolizing sugars was beneficial for lovastatin synthesis, glucose was instantly consumed as a readily available primary carbon source, therefore, influenced yield of lovastatin (Hajjaj *et al*., 2001; Kumar *et al*., 2000 and Charkravarti and Sahai 2004). In order to improve carbon to nitrogen ratio, peptone was supplemented with glucose that also positively influenced lovastatin yield.

***Effect of glycerol on lovastatin production***

In this study, we used glycerol as additional carbon source to achieve optimized lovastatin yield. Different glycerol concentrations (0.67 to 6.67%) were supplemented with wheat bran along with LPM. Sample containing all components except glycerol was used as control. It was noticed that the lovastatin yield was 1670mg/kg on supplementing of 0.67% glycerol (fig. 3). The rise in lovastatin yield may be attributed to rapid and slow metabolizing carbon sources. Since glucose is rapid metabolizing source it was rapidly utilized for fungal biomass formation. Lactose and glycerol are comparatively slow metabolizing carbon sources and are consumed after glucose depletion in polyketide

biosynthesis. For lovastatin biosynthesis, carbon repression is required which is acquired by catabolic repression or carbon starvation (Hajjaj *et al*., 2001). By further increase in glycerol concentration, carbon starvation cannot be achieved which caused decrease in lovastatin yield. Previously, researchers used different carbon sources for optimum lovastatin production (Kumar *et al*., 2000; Reynolds and Demain 1997; Szakacs *et al*., 1998 and Manzoni *et al.,* 1999), however, Xu *et al*., (Xu *et al*., 2005) and have reported two fold increase in yield of lovastatin in *Monascus ruber* by using glycerol as carbon source in SSF. Antonio *et al*., (Antonio *et al*., 2013) used crude glycerol as carbon source and found substantial yield of lovastatin by *Aspergillus terreus*.

***Effect of soybean as additional complex nitrogen source on lovastatin yield***

In this experiment effect of complex nitrogen source generated from soybean powder, was studied on lovastatin production, which caused a substantial increase in lovastatin yield. In SSF batch culture experiments basal media (wheat bran and LPM), containing nitrogen sources i.e., peptonized milk, corn steep liquor and yeast extract was used as control. Effect of additional nitrogen source, soybean powder (3.33% to 16.66%), was studied on lovastatin production. Approximately three -fold increase in lovastatin yield was observed, compared to control group. A total amount of 2860mg/kg lovastatin was produced by the addition of 16.66% soy-powder (fig. 4), on further addition of soy-powder lovastatin yield was decreased (not shown here). This increase in lovastatin yield was attributed to the addition of complex nitrogen source, soybean powder, glucose, lactose and other nutrients that help in maintaining carbon to nitrogen (C: N) ratio of media, thereby provide better conditions for lovastatin production (Casas-Lopez 2004; Bizukojc and Ledakowicz, 2008). Influence of inorganic nitrogen sources on lovastatin production was studied by some other researchers and noticed that ammonia (Andridnopoulos *et al*., 1998), urea and nitrates (Garrett *et al*., 1978) were consumed as nitrogen sources by some fungi in nitrogen metabolism. In *Aspergillus terreus* inorganic nitrogen sources e.g., ammonium (amm.) acetate, amm. tartarate, amm.nitrate, and sodium nitrate do not play important role in lovastatin production rather they were consumed for biomass formation (Hajajj *et al*., 2001). Amino acids like glycine, arginine and isoleucine were consumed as nitrogen sources by *Aspergillus terreus* but biosynthesis of lovastatin was very low (less than 1 mg per liter). Glutamate, on the other hand, showed favorable results due to its fast assimilation. Hajjaj and coworkers studied consumption patterns of C and N sources for lovastatin biosynthesis and noticed that complex nitrogen sources were consumed when assimilation of carbon sources exhausted for lovastatin production (Hajajj *et al*., 2001). Similarly some reports revealed that compactin synthesis by *Pencillium citrinum* was influenced by complex nitrogen sources (Chakravarti and Sahai 2004).

***Quantification of lovastatin by HPLC***

Reverse phase HPLC analysis was performed for the separation and quantification of lovastatin produced by SSF from *Aspergillus terreus*. Standards of lactone and hydroxyl forms of lovastatin were prepared as per methods prescribed in literature (Friedrich *et al*., 1995), and were then analyzed by HPLC (fig. 5). The retention times of hydroxyl-form and lactone-form of lovastatin were observed as 9.75 and 12.85 minutes, respectively. It was found that in SSF extracted samples, both lactone and hydroxy forms of lovastatin were produced simultaneously in variable quantities.

***FT-IR analysis of lovastatin***

FT-IR spectrum of purified lovastatin illustrated characteristic peaks at 3570 (O-H), 3104 (=C-H), 2967, 2876 (C-H), , 1705 (C=O), 1637 (C=C), 1454 ($CH_3$), 1381 ($CH_2$), 1221 (C-O, ester group) and 1122, 1064 (C-O, alcoholic group) $cm^{-1}$ which are in close accordance with the FT-IR spectrum of standard lovastatin (fig. 6). This confirmed the presence of lovastatin, produced in fungal biomass that was extracted, purified and analyzed.

***LC MS analysis of lovastatin***

Lovastatin samples extracted from biomass produced by SSF were analyzed by reverse phase LC-MS (+ESI scan). The most abundant pseudomolecular ion peak was detected at *m/z* 405.26 $[M+H]^+$ in the MS spectrum, along with other ions as sodium (*m/z* 427.24, $[M+Na]^+$) and ammonium (421.26 $[M+NH_3]^+$ ) adducts (fig. 7). The detected adduct ions of sodium and ammonium could be attributed to the reaction of lovastatin with the heated capillary in the ion source. Alakhali and coworkers developed LC-MS method for determination of simvastatin in human plasma by using lovastatin as internal standard and detected it at *m/z* 405 as protonated ion (Alakhali 2014).

## DISCUSSION

In the present study, fungal biomass was manipulated by the addition of natural carbon and various nitrogen sources to a chemically defined nutritional media at specific pH and temperature. This played significant role in maintaining carbon to nitrogen ratio, thereby consequent enhancement of lovastatin yield was observed. Different parameters were investigated for optimum lovastatin production, including pH, basal media components, additional carbon and nitrogen sources etc. Product was purified by preparative RP-HPLC, and analyzed on FT-IR and LC-MS.

Composition and constitution of culture media plays vital role in the biosynthesis of metabolites (Gallo and Katz

1972; Demaim *et al.,* 1979; Martin *et al.,* 1982; Casas-Lopez 2004 and Xu *et al.,* 2005). Mainly carbon and nitrogen sources, acting as precursors and co-factors, are involved in the formation of fungal biomass and metabolite production (Molina and Chisti 2003; Bizukojc and Ledakowics, 2007; Lai *et al.,* 2003 and Casas-Lopez *et al*., 2004), attributed by complicated regulation of gene expression and enzymes involved in polyketides synthesis (Jia *et al*., 2010). Since fungal growth takes place in two phases i.e., tropophase, rapid growth phase and idophase, metabolite production phase for development of biomass, therefore, metabolites production may vary in different provided media (Feng and Leonard 1998 and Shim, 1999). In two different studies, researchers analyzed the effect of different nitrogen and carbon sources and concluded that some C sources like fructose, lactose and glycerol slowly produced lovastatin (Lai *et al*., 2003 and Casas-Lopez *et al.,* 2004). This effect was studied both in the batch cultures, continuous fed batch cultures and discontinuous fed batch cultures (Kumar, 2000 and Bizukojc and Ledakowics, 2007b). It was noticed that fed batch cultures enhanced lovastatin yield (Novak, 1997). Both hydroxy and lactone forms lovastatin were produced by SSF as investigated by high performance liquid chromatography (HPLC), FT-IR and LC-MS. The study revealed that *A. terreus* PU-PCSIR-1 has been proved to be a potent strain for the production of lovastatin.

## CONCLUSIONS

*Aspergillus terreus* PU-PCSIR-1, a domesticated fungal isolate was found very potent strain for lovastatin production, a member of one of the most successful group of cholesterol lowering drugs. Different optimized parameters for lovastatin production were pH 5.5, temperature 28°C and 14 days of incubation in the presence of different natural carbon and nitrogen source like glycerol, soy-powder etc supplanted with additional media components, which yielded of 2860mg/Kg of total lovastatin. The findings described here for lovastatin production purification and characterization might have decent importance in domestic pharmaceuticals.

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## REFERENCES

Alakhali MK (2014). Determination of simvastatin in human plasma using liquid chromatography-mass spectrometry. *Int. J. of Pharm. & Bio. Sci.*, **5**(1): 158-65.

Alberts AWV, Huff J, Chen J, Kuron G, Hunt Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan G, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J and Springer J (1980). Mevinolin: A highly potent competitive inhibitor of hydroxy methylglutaryl coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl. Acad. Sci.* USA., **77**: 3957-3961.

Andrianopoulos A, Kourambas S, Sharp JA, Davis MA and Hynes MJ (1998). Characterization of the *aspergillus nidulans* mnr. A gene involved in nitrogen metabolite repression. *J. Bacteriol*., **180**(7): 1973-1977.

Antonio BS, Abd R, Hafiz M, Solchenbach S, Montoya A, Rollin AP, Siringan MAT and Abbas A (2013). Biodiesel- derived crude glycerol for the fungal production of lovastatin. *In*: Chemeca 2013 (41st: 2013: Brisbane, Qld.). Chemeca 2013: Challenging Tomorrow. Barton, ACT: Engineers Australia, 2013: 657-662.

Balakrishna K and Pandey A (1996). Production of biological active secondary metabolites in solid state fermentation. *J. Sci. Id. Res*., **55**: 365-372.

Balasubramaniam S, Goldstein JL and Brown MS, (1977). Regulation of cholesterol synthesis in rat adrenal gland through coordinate control of 3-hydroxy 3-methylglutaryl coenzyme A synthase and reductase activities. *Proc. Natl. Acad. Sci*., **74**: 1421-1425.

Barrios-Gonazalez J and Miranda (1996). Secondary metabolites production by solid state fermentation. *Biotechnol. An. Rev*., **2**: 85-121.

Barrios-Gonzalez J and Miranda RU (2010). Mini review on biotechnological production and applications of statins. *Appl. Mic. Biotechnol*., **85**: 869-883.

Bergstrom JD, GA, Edwards PA and Edmond J (1984). The regulation of acetoacetyl- Co A synthetase activity by modulators of cholesterol synthesis in vivo and the utilization of acetoacetate for cholesterogensis. *J. Biol. Chem*., **259**: 14548-14553.

Bizukojc M and Ledakowicz S (2007b). Simultaneous biosynthesis of (+)-geodin by a lovastatin producing fungus *Aspergillus terreus* in batch and fed-batch culture in the stirred tank bioreactors. *J. Biotechnol*., **132**: 453-460.

Bizukojc M and Ledakowicz S (2007a). A macro kinetic modelling of the biosynthesis of lovastatin by *Aspergillus terreus*. *J. Biotechnol*., **130**: 422-435.

Bizukojc M and Ledakowicz S (2008). Biosynthesis of lovastatin and (+)-geodin by *Aspergillus terreus* in batch and fed-batch culture in the stirred tank bioreactor. *Biochem. Eng. J*., **42**: 198-207.

Brown MS and Goldstein JL (1980) .Multivalent feedback regulation of HMGCoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. A receptor-mediated pathway for cholesterol homestasis. *J. Lipid Res.,* **21**(5): 17-505.

Casas-Lopez JL, Sanchez-Perez JA, Fernandez-Sevilla JM, Acien-Fernadez FG, Molina-Grima E and Chisti Y (2004). Fermentation optimization for the production of lovastatin by *Aspergillus terreus*: Use of response surface methodology. *J. Chem. Tec. Biotechnol*., **79**: 1119-1126.

Chakravarti R and Sahai V (2004). Compactin-A review. *Appl. Microbiol. Biotechnol*., **64**: 618-624.

Demain AL, Kennel YM and Aharonowitz Y (1979). Carbon catabolic regulation of secondary metabolism. Microbiol Biotechnol: Current status, future prospects. *Symp. Soc. Gen. Microbiol*., **29**: 168- 185.

Endo A (1979). Monacolin K, a new hypocholestroleimic agent produced by a *Monascus* sp. *J. Antibiot*., **32**: 852-854.

Endo A, Kuroda M and Tsujita Y (1976a). ML-236 A, ML-236B and ML-236 C, new inhibitors of cholesterogensis produced by *pencillium citrinum*. *J. Antibiot*., **32**:1346-1349.

Feng GH and Leonard TJ (1998). Culture conditions control expression of the genes for aflatoxin and sterigmatocystin biosynthesis in *Asergillus parasiticus* and *A. nidulans*. *Appl. Environ. Microbiol*., **64**: 2275-2277.

Friedrich J, Zuzek M, Bencina M, Cimerman A, Strancar A and Radez I (1995). High performance liquid-chromatographic analysis of mevinolin as mevinolinic acid in fermentation broths. *J. Chromatogr. A.,* **704**(2): 363-367.

Gallo M and Katz E (1972). Regulation of secondry metabolite biosyntehsis: Catabolite repression of phenoxazine synthase and actinomycin formation by glucose. *J. Bac*., **109**: 659-667.

Garrett, RH and Amy NK **(**1978). Nitrate assimilation in fungi. *Adv. Microb. Physiol*., **18**: 1-65.

Grundy SM (1978). Cholesterol metabolism in man. *West. J. Med*., **128**: 13-25.

Hajjaj H, Niederberger P and Duboc P (2001). Lovastatin biosynthesis by *Aspergillus terreus* in a chemically defined medium. *Appl. Environ. Microbiol*., **67**: 2596-2602.

Holker U and Lenz J (2005). Solid-state fermentation-are there any biotechnological advantages? *Current opinion in Microbio*, **8**: 301-306.

Jahromi, FM, Liang JB, Ho YW, Mohamad R, Goh YM and Shokryazdan P (2012). Lovastatin Production by *Aspergillus terreus* Using Agro-Biomass as Substrate in Solid State Fermentation. *J. Biomed. Biotechnol.*, 2012: 196264.

Jia Z, Zhang X, Zhao Y and Cao X (2010). Enhancement of lovastatin production by supplementing polyketide antibiotics to the submerged culture of *Aspergillu terreus*. *App. Biochem. and Biotech.,***160**(7):2014-2015.

Khanicheh E, Mitterhuber M, Xu L, Haeuselmann SP, Kuster GM and Kaufmann BA (2013). Noninvasive ultrasound molecular imaging of the effect of statins on endothelial inflammatory phenotype in early atherosclerosis. *PLoS One*, **8**(3): e58761.

Kumar MS, Jana SK, Senthil V, Shashanka V, Kumar SV and Sadhukhan (2000). Repeated fed-batch process for improving lovastatin production. *Process Biochem*., **36**: 336-368.

Kysilka R and Kren V (1993). Determination of Lovastatin (mevinolin) and mevinolinic acid in fermentation liquids. *J. Chromatogr.,* **630**: 415-417.

Lai LST, Pan CC and Tzeng BK (2003). The influence of Medium design on lovastatin production and pellet formation by *Aspergillus terreus* in submerged cultures. *Process Biochem.,* **38**(9): 1317-1326

Madan M and Thind KS (1998). Physiology of fungi. A.P.H. Publishing Corporation, New Delhi p.51.

Manzoni M and Rollini M (2002). Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. *Appl. Microbiol. Biotechnol.,* **58**: 555-564

Manzoni M, Rollini M, Bergomi S and Cavazooni V (1999). Production of statins by filamentous fungi. *Biotechnol. Lett*., **21**(3): 253-257.

Manzoni M, Rollini M, Bergomi S and Cavazooni V (1998). Production and purification of statins from *Aspergillus terreus* strains. *Biotechnol. Tech*., **12**: 529-532.

Martin JF, Revilla G, Zanaca DM and Lopez-Nieto MJ (1982). Carbon catabolite regulation of penicillin and cephalosporin biosyntehsis. *In*: Umezawa, H. *et al* (eds) Proceedings of symposium Trends in Antibiotic Research. Japan Antibiotic Research Association, Tokyo, pp.258-268.

Molina GE and Chisti Y (2003). Production of lovastatin by *Aspergillus terreus*: effects of C: N ratio and the principal nutrients on growth and metabolite production. *Enzyme Microb. Tech*., **33**: 270-277.

Negishi S, Huang ZC, Hasumi K and Murakawa S (1986). Productivity of monacolin K (mevinolin) in the genus Monascus. *J. Ferment. Eng*., **64**: 509-511.

Novak N, Gerdin S and Berovic M (1997). Increased lovastatin formation by *Aspergillus terreus* using repeated fed-batch process. *Biotechnol. Lett*., **19**: 947-948.

Panda BP, Javed S and Ali M (2009). Statistical analysis and validation of process parameters influencing lovastatin production by *Monascus purpureus* MTCC 369 under solid state fermentation. *Biotechnol. Bioprocess Eng*., **14**(1): 123-127.

Panda BP, Javed S and Ali M (2010). Optimization of fermentation parameters for higher lovastatin production in red mold rice through co-culture of

*Monascus purpureus* and *Monascu sruber*. Food *Bio Tech*., **3**(3): 373.

Pappu AS and Illingworth DR (1989). Contrasting effects of lovastatin and cholestyramine on low-density lipoprotein cholesterol and 24-hour urinary mevalonate sex creation in patients with heterozygous familial hypercholesterolaemia. *J. Lab. Clin. Med.,* **114**: 554-662.

Prabhakar M, Lingappa K, Babu V, Amena S, Vishalakshi N and Mahesh D (2012). Characterization of physical factors for optimum lovastatin production by *Aspergillus terreus* KLVB28mu21 under solid state fermentation. *Journal of Recent Advances in Applied Sciences*, **27**: 01-05.

Reynolds KA and Demin AL (1997). Rapamycin, FK506 and ascomycin related compounds. *In*: Strohl, W. R. (ed). Biotechnology of Antibiotics, 2nd ed., Dekker, New York, p.497.

Robinson T, Singh D and Nigam P (2001). Solid-state fermentation a promising microbial technology for secondary metabolite production. *Appl. Microbiol. Biotechnol*., **55**: 284-289.

Rodriguez Porcel EM, Casas Lopez JL, Sanchez Perez JA and Chisti Y (2007). Enhanced production of lovastatin in a bubble column by *Aspergillus terreus* using a two stage feeding strategy. *J. Chem. Technol. Biotechnol*., **82**: 58-64.

Rodwell VW, Nordstrom JL and Mitschellen JJ (1976). Regulation of HMGCo A reductase. *Adv. Lipid Res*., **14**: 1-74.

Shaligram NS, Singh SK, Singhal RS, Szakacs G and Pandey A (2008). Compactin production in solid-state fermentation using orthogonal array method by *P. brevicompactum*. *Biochem. Engg. J.,* **41**(3): 295-300.

Shim WB and Woloshuk C P (1999). Nitrogen repression of fumonisim B1 biosynthesis in *Gibberella fujikuroi*. *FEMS Microbiol. Lett*., **177**(32): 109-116.

Springer J (1980). Mevinolin: A highly potent competitive inhibitor of hydroxymethylglutaryl-Coenzyme A reductase and a cholesterol lowering agent. *Proc. Natl. Acad. Sci*., U.S.A., **77**: 3957-3961.

Suryanarayan S (2003). Current industrial practice in solid state fermentations for secondary metabolite production: The Biocon India experience. *Biochem. Eng. J*., **13**: 189-195.

Szakacs G, Morovjan G and Tengerdy RP (1998). Production of lovastatin by a wild strain of *Aspergillus terreus*. *Biotechnol. Lett*., **20**: 411-415.

Thomas L, Larroche C and Pandey A (2013). Current developments in solid-state fermentation. *Biochem. Eng. J.*, **15**(81): 146-161.

Tobert JA (1987). New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl coenzyme A reductase. *J. AHA Circ*., **76**: 534-538.

Tobert JA (2003). Lovastatin and beyond: The history of HMGCo-A reductase inhibitors. *Nat. Rev. Drug. Discov*., **2**(7): 517-526.

Valera HR, Gomes J, Lakshmi S, Gururaja R, Suryanarayan S and Kumar D (2005). Lovastatin production by solid state fermentation using *Aspergillus flavipes*. *Enzyme and Microb. Tech*. **37**(5): 521-526.

Xu BJ, Wang QJ, Jia XQ and Sung CK (2005). Enhanced Lovastatin production by solid state fermentation of Monascus ruber. *Biotech and bioprocess Engg*., **10**: 78-84.