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Mini Review

Solid state fermentation: An effective method for Lovastatin production by fungi over submerged fermentation

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The Enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase) catalyses conversion of HMG CoA to mevalonate during cholesterol biosynthesis. Lovastatin is used as an anti-cholesterol drug which blocks HMG CoA reductase activity. Lovastatin has been reported to be produced by Submerged Fermentation (SmF) and Solid State Fermentation (SSF) by fungi. Of-late it is used not only as anti-cholesterol drug but as anti-inflammatory agent, cancer cell apoptosis, restoration of renal function, bone disorders treatment; immune-modulatory role is also being investigated. This review provides insight into the different lovastatin production strategies employed by SSF, its advantages over SmF, optimisation parameters, lovastatin genetics etc.

Key words: HMG CoA. Lovastatin, Fungi, SSF, SmF, Pleiotropic.

INTRODUCTION

The World Health Organization estimated that 17.3 million lives were lost in 2008 and an expected 23.6 million people will die of cardiovascular diseases (CVD) by the year 2030 (WHO, 2011). Close to 80% of mortality rates were reported from the lower and middle income countries. Hypercholesterolemia is one of the reasons for these deaths. The treatment of the hypercholesterolemia is targeted by decreasing the low density lipoprotein (LDL) cholesterol and is best achieved by medications when diet and exercise fail (Lloyd-Jones et al.,2009) . A wide variety of biologically active compounds are produced by fungi, a large proportion of which are produced by the polyketide biosynthetic pathway. Fungal polyketides represent structurally diverse group and with many displaying important biological activities such as antibiotic and other related pharmacological properties (Bedford et al., 1995). Noted among the fungal metabolites are statins (anti-cholesterol compounds) that are considered as the most important class produced by the polyketide pathway. Statins comprises of Compactin, lovastatin, pravastatin, simvastatin, rosuvastatin, atorvastatin and fluvastatin. Compactin and lovastatin are of biological origin whereas simvastatin, pravastatin, rosuvastatin etc are chemical modifications of compactin and lovastatin (Chakravarti and Sahai, 2004). The biosynthetic pathway involved in statin production starts from acetate units linked to each other in a head to- tail fashion to form a polyketide chain. Lovastatin (Figure 1) prevents formation of mevalonate from HMG Co-A (Sreenivasan et al., 2008; Marcin and Stanislaw, 2009) by competitively inhibiting the enzyme HMG CoA Reductase. Lovastatin can exist in two forms i.e hydroxyl from and lactone form, of which the hydroxyl-form is the active drug. The lactone forms (e.g. lovastatin, simvastatin, cerivastatin) are lipophilic while the acid

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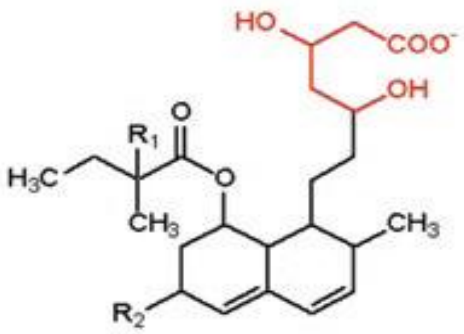

Figure 1. Structure of Lovastatin

forms are hydrophilic for example atorvastatin, fluvastatin, pravastatin (Zamvil and Steinmann., 2002).

Lovastatin is obtained from different genera and species of filamentous fungi. Several fungal genera including *Aspergillus, Penicillium, Monascus, Paecilomyces, Trichoderma, Scopolariopsis, Doratomyces, Phoma, Phythium, Gymnoascus, Hypomyces* and *Pleurotus* are reported as lovastatin producers (Bizukojc and Stanislaw, 2009, Cabral et al., 2010, Srinu et al., 2010). In addition even the Basidiomycetes mushrooms have been recently reported to be used. *Pleurotus* spp and its related strains produce higher concentrations of lovastatin (Alarcon et al., 2003). Recently, a marine actinomycete has also been reported to produce lovastatin (Srinu et al., 2010). This review gives an insight into lovastatin production by SSF in the last 5-6 years and the possible multiple (pleiotropic effects) applications of lovastatin in medical field.

HISTORY

A US based company (in 1950's), Wm. S. Merrell Co., reported a compound with anti-cholesterol property, called as Triparanol (MER/ 29) which blocked the conversion of desmosterol to cholesterol. Later it was reported that triparanol was an ineffective drug and associated with lens cataracts, hair loss in rats and dogs, also higher doses causes blindness in rats (Steilberg, 2006). This led to ban on triparanol. Masao Kuroda and Akira Endo, supported by their team, at Sankyo Co. Tokyo, screened numerous fungi and discovered that *Penicillium citrinum* produced anti-cholesterol compound. This compound from *P. citrinum* was christened as ML236B (referred to as compactin). With several tests the carcinogenic effects of compactin came to light due to noted lymphomas in dogs treated with higher doses of compactin. This led to the said company to halt proposed clinical trials. Later, Merck, a competitor, reported the production of Mevinolin (Lovastatin) from *Aspergillus terreus*. It was later discovered that in fact what they referred to as lymphomas associated with compactin were actually the accumulation of reductase proteins in endoplasmic reticulum in response to statin therapy., Merck got the approval from Food and Drug Administration (FDA) in 1987 to release Mevinolin into the market. However, Akira Endo in 1989, reported that *Monascus sp* also produced reductase inhibitors and subsequently obtained a patent (Steilberg, 2006; Endo, 2008).

Pleiotropic Effects of Lovastatin

The clinical applications of lovastatin have been well documented (Sreenivasan et al., 2008). It finds its possible applications for more medical uses such as reducing instances of peripheral vascular diseases, prevention of strokes, stabilization of artheromatous plaques, improved endothelial functions and prevention of thrombus formation (Tandon et al., 2005; Barrios and Miranda, 2010).

Alzheimers Disease **(AD):** Amyloid plaque formation in brain is noted in patients with AD due to production of neurotoxic amyloid protein (produced by alternate processing of amyloid precursor protein). Animal and cell culture experiments reported decreased prevalence of AD on lovastatin treatment (Eckert et al., 2005). Human trials did not yield 100% results and further work needs to be warranted in the same area to attribute exact role of lovastatin (Eckert et al., 2005).

Multiple Sclerosis **(MS)**: Lovastatin supressed production of tumor necreosis factor- α(TNF-α) by Interferon-α (IFN-α). A decreased level of inflammatory response and protection of hosts cellular damage was noted. Lovastatin also inhibited major histocompatibiliy complex-II (MHC-II) upregulation in antigen presenting cells (APC's). Atorvastatin prevented or reversed paralysis in murine experiemental autoimmune encephalitis (EAE) models thus indicating the immunomodulatory role of lovastatin (Zamvil and Steinmann, 2002).

Bone Disorders: In murine models prolonged infusions or large doses of lovastatin stimulated bone formation both *in-vivo* and *in-vitro* (Sreenivasan et al., 2008). Pre-clinical studies reported that nano particle delivery of lovastatin may fasten human bone fractures (Garrett et al., 2007). This positive effect may also aid in treatment

of osteoporosis (Sreenivasan et al., 2008).

Renal Protection: Glomerulonephritis associated kidney damage may be retarded by lovastatin treatment. This may be possibly due to down regulation of inflammatory cytokines and activity of GTPases Ras superfamily. The exact role of statins is yet to be elucidated (Buemi et al., 2002).

Cancer: Induction of apoptotic response in human acute myeloid leukemia (AML) cells was noted on lovastatin treatment (Xia et al.,2001). Inhibition of geranylgeranylation of target proteins is the mechanism of lovastatin-induced apoptosis in AML cells. The antiproliferative properties of lovastatin may be used as an effective anticancer drug (Tandon et al., 2005). The mechanism underlying lovastatin induced apoptosis of malignant cells remains unclear (Bonovas et al., 2006; Glynn et al., 2008). About 20 %– 55% reduction in site-specific cancers (colorectal, breast, prostate, lung and pancreatic) was observed with the use of statin therapy (Glynn et al., 2008). Inhibition of Ras farsenylation is associated with reduction and proliferation of cancer in human glioblastoma cells (Xia et al., 2001). Further studies need to be carried out to exactly elucidate if statins can be used as anti-cancer drug as concordant results have not been obtained (Sreenivasan et al., 2008).

Rheumatoid Arthritis **(RA)**: Immunomodulation and modified endothelial function by lovastatin may aid to decrease problems associated with RA (Sreenivasan et al., 2008).

A recent report suggests the role of statins as an immuno-modulator in treatment of vitiligo (Sreenivasan et al., 2008)and its beneficial part in graft transplant is being investigated (Sreenivasan et al., 2008). With its multiple applications, statins in future may play a pivotal role in medico-pharmaceutical field (Tandon et al., 2005).

Biosynthesis and Genetics of Lovastatin Production

The lovastatin pathway initiates from assembly of acetate units in head to tail fashion to form the two polyketide chains i.e Lovastatin Nonkedtide Synthase (LNKS) and Lovastatin Diketide Synthase (LDKS) (Barrios et al., 2010). The first chain is Monacolin J (assembled form 2 acetate units and 9 methionine units) which is later attached to the second chain i.e 2 Methyl butryl CoA (assembled from 2 acetate units and 1 methionine unit) lovastatin. The genes involved are *lov* B, *lov* C, *lov* F and *lov* D. The multifunctional Polyketide Synthase (PKS) system encodes the LNKS and LDKS in *A terreus*. LNKS is a gene product of *lov* B, interacts with *lov* C (a putative enoyl reductase) to form the dihydro monacolin L which is later converted to Monacolin J. The LDKS, gene product of *lovF*, interacts with *lov D* (transesterase enzyme) that catalyzes the attachment of the 2-methylbutyric acid to monacolin J to form the functional lovastatin (Manzoni and Rollini, 2002, Sreenivasan et al., 2008, Barrios and Miranda, 2010).

In tandem to this works on gene mutation and cloning of lovastatin genes into other hosts have also been reported (Pfeifer and Khosla, 2001, Xie and Tang, 2007). The possibility of conversion of Monacolin J (MCJ) to simvastatin (a semisynthetic form of lovastatin) using *lov* D gene that has been transferred to *E coli* has been reported (Xie and Tang, 2007). The substrate used for the *lov* D was α-dimethylbutyryl-S-NAC (or α-dimethylbutyryl-S-methylthioglycolate). However, this reaction resulted in poor product turnout (Xie and Tang, 2007). Later, DMB-S-methyl mercaptopropionate (DMB-S-MMP) was used as an alternative substrate. This replacement with a cheaper substrate gave a better simvastatin yield but the rate of conversion was low (<60%). Lastly, site directed mutagenesis of cysteine to alanine and aspargine yielded a double mutant of *E coli* with almost >99% transformation of MCJ to simvastatin to yield 18g/L in approximately 18 hrs (Barrios and Miranda, 2010).

Solid Substrate Fermentation for Lovastatin Production

Lovastatin production and optimisation of fermentation parameters has been of great interest since its discovery (Kumar et al., 2000, Sayyad et al., 2007, Jaivel and Marimuthu, 2010b). Many efforts and trials have been performed to increase the titre (Ferron et al., 2005, Jaivel and Marimuthu, 2010a, Prabhakar et al., 2011). Initially, all production processes were carried in Submerged Fermentations (SmF) by varying conditions of its physico-nutritional parameters. The submerged processes have not yielded constant results and higher yield and hence a shift towards to Solid State Fermentation (SSF) was gaining popularity for multiple industrially important products such as enzymes, pigments, antibiotics etc. SSF has been widely employed in industrial productions because of its advantages such as better process control, maximum substrate utilisation, lower chances of contamination, easy downstream processing etc (Pandey et al., 2001). Many bacteria and fungi have been utilised for production of industrially important products by SSF (Pandey et al., 2001)

The production of lovastatin by SmF has given varied

Table 1. Lovastatin Production by SmF

Sl No.	Microorganism	Lovastatin yield (mg/L)	References
1	*Aspergillus terreus*	0.40	Szakacs et al., 1998
2	*Aspergillus terreus*	2200	Kumar et al., 2000
3	*Aspergillus terreus*	55	Samiee et al., 2003
4	*Monascus pilosus*	725	Miyake et al., 2006
5	*Monascus purpureus*	0.318	Sayyad et al., 2007
6	*Monascus purpureus*	737	Ahmed et al., 2009

results in terms of yield (Table 1). The selection of carbon and nitrogen source in the growth medium governs the lovastatin yield. Various nutritional combinations have been reported in submerged conditions (Sayyad et al., 2007, Sorrentino et al., 2010, Osman et al., 2011,). Glucose, lactose, fructose and glycerol have been widely used as carbon source. Glucose and lactose are suited as good carbon source at low concentrations. Higher glucose concentration facilitates filamentous growth but decreases titre along with production of ethanol as by-product and increasing medium viscosity (Bizukojc and Stanislaw, 2009). So a slowly metabolizable carbon source like lactose is best suited. Glycerol is also of choice (Miyake et al., 2006). Dox medium or modified dox medium also favours lovastatin growth (Atalla et al., 2008).

Various nitrogen sources often used in organic form (peptone, soyabean, corn steep liquor) and inorganic form (ammonium sulphate, ammonium chloride) (Sayyad et al., 2007; Atalla et al., 2008) also determine the final yield.

Vis-a-vis there has been use of varied combinations of carbon: nitrogen sources with different organisms for study. Each organism utilizes C:N in varying amounts thus leading to varied results (Sayyad et al., 2007, Sorrentino et al., 2010, Osman et al., 2011).

The addition of supplements to growth medium influences the yield too. Linoleic acid, butyrolactone, dodecane, acetic acid favours a higher yield. These supplements work best at low concentrations and work either directly incorporating themselves into specific pathway or by acting as an enzyme activator (Sorrentino et al., 2010 and Osman et al ., 2011). Butyrolactone and Dodecane (2.5%) increased the yield by 3-4 fold (Bizukojc and Stanislaw, 2009). Sodium acetate supplementation triggers better yield by acting as a precursor for statin synthesis (Osman et al., 2011).

Lovastatin production also has been carried by SSF approaches with promising results. The results were far surprising that expected with high titre of 1110µg/gm dry weight of substrate, thus portraying immense potentials of SSF over SmF. Numerous data is present regarding lovastatin production by SmF and optimisation parameters reported. The production of lovastatin by solid state fermentation (SSF) has gained popularity as it involves lower media cost, stability of the product, increased yield and better substrate porosity (Chanakya et al., 2011; Reddy et al., 2011).

The substrates used in fermentation include wheat bran, rice bran, non-glutinous rice, orange peels, grain husks (Reddy et al., 2011; Jaivel and Marimuthu, 2010a; Chankya etal., 2011). Wheat bran topped the list as the best substrate, with a maximum yield of 3273.4 µg/g (Panasuriya and Singhal., 2010) and a yield range of 806 mg/L to 982.3 mg/L (Jaivel and Marimuthu, 2010a). *Per se*, an increase or decrease in moisture content affects the oxygen and water balance (Panasuriya and Singhal., 2010) and decreases lovastatin yield. The higher yield associated with SSF is primarily due to increased mycelial density provided with an optimum moisture range between 60%-70% (Prabhakar et al., 2011). Lovastatin yield of 730 µg/g of dry weight substrate i.e wheat bran at a moisture level of 65% and temperature of 30 ^{O}C has been reported using mutated strain of *A terreus* (Prabhakar et al., 2011). Rice bran was of good choice specially when supplemented with nitrogen source (as rice is poor in nitrogen source) and better compared to rice husk (Pie-Lien et al., 2007). Low protein content may be dealt by addition of peptone in substrate. Rice based unsupplemented medium yields 1.703 mg/g lovastatin using *Monascus purpureus* MTCC 369. Rice supplemented with soya bean powder, sucrose and yeast extract inoculated with *M. ruber* also resulted in high titre (Xu et al., 2005).

Selection of glucose (repressive) along with non-repressive carbon source in combination yields increased lovastatin titre in *M. pilosus*. Glucose and maltose are the best known carbon sources (444 mg/L). Glucose is said purported to exert repressive action but a combination of glucose, glycerol and peptone in medium is best for *M. pilosus* to produce lovastatin (Miyake et al., 2006). Dextrose, KH_2PO_4 and $FeSO_4$ do not aid much in

lovastatin production when compared to NH_4Cl, $MgSO_4$ and NaCl when present in medium inoculated with *M. purpureus* 369 (Panda et al, 2009b). Various physico-nutritional parameters that govern lovastatin production have been well documented (Sayyad et al., 2007, Marcin and Stanislaw, 2009, Sorrentino et al., 2010, Osman et al., 2011).

Presence of one amino acid is mandatory in the growth media. Riboflavin, pyridoxine and calcium pantothenate when used as supplements invariably increased yield, except for thiamine. Methionine is suited as it is directly involved in biosynthetic pathway and gives a yield of 180 µg/ml (Marcin and Stanislaw, 2009). It is necessary to maintain a striking balance between carbon and nitrogen source for obtaining a desirable lovastatin production as they regulate the biomass and metabolite production (Osman et al., 2011).

Incorporation of various supplements to the growth medium has been studied (Marcin and Stanislaw, 2009) . Addition of tween-80 increases the yield whereas $ZnSO_4$ had no effect on yield, but $MgSO_4$ decreased yield by 4.11% (Danuri, 2008). Addition of acetic aci at range of 0.1% -0.3% also favoured good yield. Glycerol (3%), $NaNO_3$ (0.2%) contributed in higher yield. A glycerol level above 0.3% decreased titre as it affected fungal cell perme-ability (Xu et al., 2005).

The use of agro-based wastes (wheat bran, corn hull and rice husk), fruit wastes (sugarcane bagasse, orange peel and orange pulp) and their combination has been studied (Prahbakar et al., 2011, Reddy et al., 2011, Panasuriya and Singhal., 2010]. Addition of nutrient medium to dried substrate has also been the subject of study with contradictory results. Lower yield of lovastatin is noted when glucose, lactose, sucrose was incorporated into solid substrate (Panasuriya and Singhal, 2010). Glucose, lactose and sucrose when added to Dried Fermented Matter (DFM) tend to decrease the yield of lovastatin to 2101±51, 2534±29 and 2435±38 µg g^{-} respectively. However, contradictorily a higher yield was recorded when sweet sorghum syrup is incorporated with nutrient solution (Jaivel and Marimuthu, 2010a).

Mutation by Ethyl Methyl Sulphonate (EMS) and UV of *A terreus* KLV28mu21 recorded higher yield (Prabhakar et al., 2011). *A terreus* isolated from contaminated oyster mushroom bed subjected to EMS and UV mutation (strains JPM-EMS2and JPM-UV2) produced lovastatin in better appreciable quantity (948.50mg/L and 1553.02 mg/L) (Jaivel and Marimuthu, 2010a).

Response Surface Methodology (RSM) approach has been well adopted to assess lovastatin yield and determine the optimum fermentation parameters (Sayyad et al., 2007). A temperature of $29.46^{O}C$, fermentation time of 14.43 days with an initial in-oculum level of 5 ml at pH 6.00 yields 3.432 mg/g (Panda et al., 2009a) of lovastatin using *M purpureus* MTCC 369 under SSF. Fermentation studies have been revealed the temperature range of $28^{O}C$ -$30^{O}C$ as the best suited temperature and at a pH range between 5-6, while for *A fischerii* its relative humidity is of 60%, pH 5, and temperature set at $30^{0}C$ with lactose and malt extract as its main optimum is best suited for a maximum yield (Chanakya et al. ,2011). SSF holds true potential for production of many industrially important products (Pandey et al., 2001). The limitations that may be encountered in SSF are in controlling of process parameters and scale up from laboratory to exploitable industrial level (Mienda et al., 2011).

The higher lovastatin production in SSF is primarily related to enhanced transcriptional rates of biosynthetic genes *lov* E and *lov* F resulting in yield increase by 4.6 fold and 2 fold respectively. The *lov* E and *lov* F transcrips accumulation was 20 and 6 fold lower than in SSF when a liquid medium (SmF) of identical concentration when used. Genetically engineered *A terreus* could synthesise 2,2 dimethyl butyrate (side chain of simvastatin) and produce simvastatin directly rather than lovastatin (Barrios-González et al., 2008). By isolating DNA, RNA and analysing them by corresponding blotting techniques it was confirmed that during SSF the gene transcript levels of lov genes (*lov* B and *lov* F) was higher when compared to SmF meanwhile the expression of gldB (NADP dependent glycerol dehydrogenase), gene for osmotolerance, in SSF seem to indicate the role of osmotic genes in *A nidulans* when inert substrate polyurethane foam was used (Barrios-González et al., 2008). This study putatively substantiates the usage of SSF as a modern optimum technological approach for lovastatin production.

CONCLUSION

Lovastatin production has gained large scale importance with more emphasis on use of SSF approach. This has resulted in continual search for novel and cheaper substrates with stress on optimisation of production technology. The molecular level studies also substantiate the role of using SSF technology. With the pleiotropic effects unfolding, statins may be a master key to control or regulate many major diseases in the future

REFERENCES

Ahmed A, Panda BP, Khan A, Ali M, Javeed S (2009). Downstreaming and purification of lovastatin from *Monascus purpureus* culture. Thai J. Phar. Sci., 33: 39-46.

Alarcon J, Aguila S, Arancibia-Avila P, Fuentes O, Zomorano-Ponce E, Hernandez M (2003). Production and purification of statins from *Pleurotus ostrearus* (Basidiomycete) strains. Z

Naturforsch., 58: 62-64.
Atalla, MM, Hamed ER, El-Shami AR (2008). Optimization of a culture medium for increased mevinolin production by *Aspergillus terreus* strain. Malaysian J. Microbiol., 4(2): 6- 10.
Barrios-González J, Baños JG, Covarrubias AA, Garay-Arroyo A (2008). Lovastatin biosynthetic genes of Aspergillus terreus are differentially expressed in solid-state and in liquid submerged fermentation. Appl. Microbiol. Biotechnol., 79: 179–186.
Barrios GJ, Miranda RU (2010). Biotechnological production and applications of statins. Appl. Microbiol. Biotech., 85: 869-883.
Bedford JD, Schweizer E, Hopwood DA and Khosla C (1995). Expresssion of a fungal polyketide synthase in the bacterium *Streptomyces coelicolor* A3(2). J. Bacteriol., 177(15): 4544-4588.
Bizukojc M, Stanislaw L (2009). Physiological, morphological and kinetic aspects of lovastatin biosynthesis by *Aspergillus terreus.* Biotech J., 4(5): 1-61.
Bonovas S, Filioussi K, Tsavaris N, Sitaras NM (2006). Statins and cancer risk: a literature-based metaanalysis and metaregression analysis of 35 randomized controlled trials. J. Clin. Oncol., 24: 4808-4817.
Buemi M, Senator M, Corica F, Aloisi C, Romeo A, Cavalloro E, Flocccari F, Tramonto D, Frisina N(2002). Satins and progressive renal disease. Med. Res. Rev., 22: 76-84.
Cabral ME, Delgado OD, Sampietro DA, Catalan, Figueroa LIC, Farina JI (2010). Antifungal activity and the potential correlation with statin producing ability: An optimised screening applied to filamentous fungi from Las Yungas subtropical rainforest. Res. J. Microbiol., 5(9): 833-848.
Chakravarti R, Sahai V (2004). Compactin—a review. Appl. Microbiol. Biotechnol., 64: 618-624.
Chanakya P, Latha PM, Srikanth M (2011). Solid state fermentation for the production of lovastatin by *Aspergillus fischerii.* Res. J. Pharma. Sci. Biotech., 1(1): 9-13.
Danuri H (2008). Optimizing angkak pigments and lovastatin production by *Monascus purpureus*. Hayati J. Biosci., 15(2): 61-66.
Eckert GP, Wood WG, Muller WE (2005). Statins: drugs for Alzheimer's disease? J. Neural Transm., 112(8): 1057-1071.
Endo A (2008). A gift from nature: the birth of the statins. Nat. medicine, 14(10): 1050-1052.
Ferro´ n Vilches MA, Lo´ pez Casas JL, Perez JAS, Sevilla JMF, Christi Y (2005). Rapid screening of *Aspergillus terreus* mutants for overproduction of lovastatin. World J. Microbiol. Biotechnol., 2: 123–125
Garrett IR, Gutierrez GE, Rossini G, Nyman J, McCluskey B, Flores A, Mundy GR (2007). Locally delivered lovastatin nanoparticles enhance fracture healing in rats. J. Ortho. Res., 25(10): 1351-1357.
Glynn SA, O'Sullivan D, Eustace AJ, Clynes M, O'Donovan N (2008). The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, simvastatin, lovastatin and mevastatin inhibit proliferation and in-vasion of melanoma cells. BMC Cancer; 16: 8-9.
Jaivel N, Marimuthu P (2010a). Optimization of lovastatin production in solid state fermentation by *Aspergillus terreus.* Intl J. Engg. Sci. Technol., 2(7): 2730-2733.
Jaivel N, Marimuthu P (2010b). Isolation and screening of lovastatin producing microorganisms. Int. J. Engg. Sci. Technol., 297: 2607-2611.
Kumar MS, Jana SK, Senthil V, Shashanka V, Kumar SV, Sadhukhan AK (2000). Repeated fed-batch process for improving lovastatin production. Process Biochem; 36: 363-368.
Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, Simone GD, Ferguson BT, Ford E, Karen F, Gillespie C, Go A, Greenlund K, Hasse N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, lackland D, Lisabeth L, Marelli A, Mc Dermott HM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosawond W, Sacco R, Sorlie P, Stafford R, Thon J, Smoller SW, Wong ND, Rosett JW (2009) Heart Disease and Stroke Statistics-2010 Update: A Report From the American Heart Association., Circulation: 1-174
Manzoni M, 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.
Mienda SB, Idi A, Umar A (2011). Microbiological features of solid state fermentation and its applications- An overview. Res. Biotechnol., 2(6): 21-26.
Miyake T, Uchitomi K, Zhang M Y, Kono I, Nozaki N, Sammoto H, Inagaki K (2006). Effect of the principle nutrients on lovaststin production by Monascus pilosus. Biosci. Biotechnolm Biochem., 70(6): 1154-1159.
Osman ME, Khattab OH, Zaghlol, El-Hameed RMA (2011). Optimization of Some Physical and Chemical Factors for Lovastatin Productivity by Local Strain of *Aspergillus terreus.* Aus. J. Basic Appl. Sci., 5(6): 718-732.
Panasuriya CR, Singhal RS (2010). Response surface methodology for optimisation of lovastatin production by solid state fermentation. Braz. J. Microbiol., 41(1): 164-172.
Panda BP, Javed S, Ali M (2009a). Statistical analysis and validation of process parameters influencing lovastatin production by *Monascus purpureus* MTCC 369 under solid state fermentation. Biotech Bio-process Engg., 14: 123-127.
Panda BP, Javed S, Ali M (2009b). Engineering Rice Based Medium for Production of Lovastatin with *Monascus* Species. Czech J. Food Sci., 27(5): 352–360.
Pandey A, Soccol CR, Rodrigoez-Leon, Nigam P (2001). Solid state fermentation in biotechnology- Fundamentals and Applications Ist Edn, Asiatech Publications Inc132-133.
Pie-Lien W, Zhi-nan X, Pei-lin C (2007). Lovastatin production by *Aspergillus terreus* in solid-state fermentation. J. Zhejiang Uni. Sci., 8(9): 1521-1526.
Pfeifer BA, Khosla C (2001). Biosynthesis of Polyketides in Heterologous Hosts. Microbiol. Mol. Bio. Rev., 65(1): 106-118.
Prabhakar M, Lingappa K, Vivek B, Amena S, Vishalakshi N, Mahesh D (2011). Characterization of physical factors for optimum lovas-tatin production by *Aspergillus terreus* klvb28mu21 under solid state fermentation. J. Rec. Adv. Appl. Sci., 27: 1-5.
Reddy DSR, Latha DP, Latha KPJ (2011). Production of lovastatin by solid state fermentation by *Penicillium funiculosum* NCIM 1174. Drug Invent Today., 3(6): 75-77.
Samiee SM, Moazami N, Haghighi S, Mohseni S, Mirdamadi A, Bakhtiari MR (2003). Screening of lovastatin production by filamen-tous fungi. Iran Biomed. J., 7(1): 29-33.
Sayyad SA, Panda BP, Javed A and Ali M (2007). Optimization of nutrient parameters for lovastastin production by *Monascus*

purpureus MTCC 369 under submerged fermentation using response surface methodology. Biotechnol. Prod. Proc. Engg., 73: 1054-1058.
Sorrentino F, Roy I, Keshavarz T (2010). Impact of linoleic acid supplementation on lovastatin production in *Aspergillus terreus* cultures. Biotechnol. Prod. Proc. Engg., 88: 65-73.
Sreenivasan A, Shubahgar S, Arvindan R, Viruthagiri T (2008). Microbial production and biomedical applications of Lovastatin. Ind. J. Pharm. Sci., 70: 701-9.
Srinu M, Bhushan GVP, Moges F, Srilakshmi J, Sankar G, Prabhakar T, Lakshminarayana K (2010). Screening of HMG CoA reduc-tase inhibitor producing marine actinomycetes. J. Pharm. Sci. Health., 2(1): 66-74.
Steilberg D (2006). An interpretive history of the cholesterol controversy, part V. The discovery of the statins and the end of the controversy. J. Lipid Res., 47: 1339-1351.
Szakacs G, Morovjan G, Tengrendy PR (1998). Production of Lovastatin from a wild strain of *Aspergillus terreus.* Biotechnol. let., 20(4): 411-415.
Tandon V, Bano G, Khajuria V, Parihar A, Gupta S (2005). Pleiotropic effects of statins. Ind. J Pharmacol., 37(2): 77-85.
Xia Z, tan MM, Wing WW, Dimitroulakos J, Minden MD, Penn LZ (2001). Blocking protein geranylgeranylazation is essential for lovastatin induced apoptosis of human acute myeloid leukemia cells. Leukem., 15: 1398-407.
Xie X, Tang Y (2007). Efficient synthesis of simvastatin using whole-cell biocatalysis. Appl. Environ. Microbiol., 73:2054–2060.
Xu B, Wang Q, Jia X, Sung C (2005). Enhanced lovastatin production by solid state fermentation of *Monascus ruber*. Biotechnol. Biochem. Engg., 10: 78-84.
Zamvil SS, Steinman L (2002). Cholesterol-lowering statins possess anti-inflammatory activity that might be useful for treatment of MS. Neurol., 59: 970-1.