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Review Article

A REVIEW: LOVASTATIN PRODUCTION AND APPLICATIONS

RADHA K V*, LAKSHMANAN D

Department of Chemical Engineering, A.C.Tech, Anna University, Chennai INDIA .Email: radhavel@yahoo.com

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ABSTRACT

Cholesterol is an organic compound, which is produced in humans by a complex metabolism. It acts as a precursor for the synthesis of many steroids, vitamin D and also helps in membrane transport. Increase of cholesterol in humans, leads to Cardio Vascular Disorders and finally death. This can be reduced by inhibiting the HMG-CoA reductase, which is an important precursor in formation of mevolanate from Acetyl CoA. Inhibition is done by statin drug, which is produced by many fungi through polyketide pathway. Many fungus produce lovastatin, while other statins like rosuvastatin, simvastatin, pravastatin, fluvastatin are synthesized from lovastatin and mevastatin. Other than reduction of cholesterol, lovastatin is shown to provide various medicinal properties like anti-cancer, bone maturation, multiple sclerosis. In this review, a detailed note on various organisms employed for the production of lovastatin and different fermentation techniques for the same was studied. This review also helps in understating the inhibition of HMG-CoA reducatse and various medicinal properties of lovastatin.

Keywords: Lovastatin, Fungus, Submerged fermentation, Solid state fermentation, HMG-CoA reducatse.

INTRODUCTION

Cholesterol is an important organic chemical substance, which plays a vital role in body metabolism and membrane transport [1]. They are much essential in the membrane fluidity over the range of temperature; they also act as a precursor for synthesis of steroid hormones, bile acids and vitamin D. The cholesterol is synthesized from acetyl-Co-A in a complex 37 steps process, where the rate limiting step was the conversion of HMG-Co-A to mevolanate using HMG-Co-A reductase [2]. These cholesterols were categorized as HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein) and its optimum level should be less than 100 mg/dL and greater than 60 mg/dL respectively (National Cholesterol Education program) [3]. Increase or decrease in the level of cholesterol leads to various cardio vascular disorders like arthrosclerosis, hypercholesterolemia, myocardial infarction, atheroma, peripheral vascular diseases, and so on [4].

The World Health organization have reported that nearly 17.3 million people had died due to Cardio Vascular Disorder (CVD) in 2008 and over 80% of CVD death is common among lower and middle income countries. WHO (World Health Organization) also estimated that over 23.6 million people will die by 2030, due to CVD (WHO, 2012). CVDs are caused due to use of tobacco and hypercholesterolemia. Hypercholesterolemia is one of the major reasons for the CVDs, where cholesterol is deposited in blood vessels. Hypercholesterolemia was treated with medication to reduce the low density lipoproteins, when diet and exercise are insufficient. Triparnol is the first anti-cholesterol drug discovered in the year 1958. It reduces the cholesterol level in the blood by inhibiting demosterol thus controlling the formation of cholesterol. The drug was banned and withdrawn from market, due to its severe side effects [5]. Later a new drug ML236B (Compacitin) was discovered by Masso Kuroda and Akira Endo [6], from *Penicillium citrinum*. But this was also withdrawn during clinical trials, due to its carcinogenic effect. Later, Merck (1980) discovered an anti-cholesterol drug from *Aspergillus terrus*, which reduced blood cholesterol and also has no side effects. The drug was named as lovastatin (Mevinolin) and approved by FDA in the year 1987.

All the statin molecules reduce the cholestrol level by same mechanism, where they inhibit the HMG-CoA reductase, an important precursor in the cholesterol synthesis. Figure 1 shows the synthesis of cholesterol from Acetyl Co-A, and the inhibition mechanism by lovastatin at the rate limiting step. Lovastatin is a fungal polyketide, which has a napthelin ring and a lactone ring, where the lactone ring binds to HMG-CoA reductase enzyme and there by inhibits the formation of cholesterol 2.

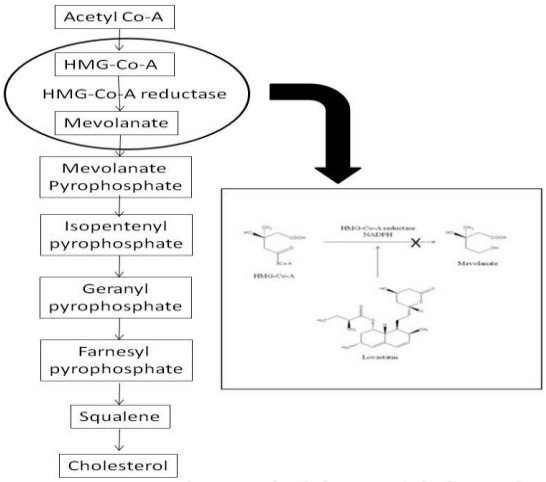

Figure 1: Bio synthesis and inhibition of Cholesterol

The statin was produced by many filamentous fungi through a fungal polyketide pathway. The biosynthesis studies of lovastatin were done on *A.terreus* strain [7,8,9]. The production starts from the acetate units in head to tail function to form two polyketide chains [10]. Of many statin molecules, lovastatin and mevastatin are produced by the fungal species, while other statins like rosuvastatin, simvastatin, pravastatin, fluvastatin, atrovastin, cerivastatin, are produced semi-synthetically from lovastatin [11]. This review gives a detailed study on various organisms that produce lovastatin, through various fermentation processes, and also the wide range of medicinal application of it.

Production of Fungal Lovastatin

Lovastatin was the first statin drug, which was approved by United States Food and Drug Administration in the year 1987 11. *A.terreus* is a strain that was used in the production of lovastatin, which was widely studied and an imminence research is being carried out [12, 13]. Various *Monascus sp.* had reported to produce lovastatin and other statin compounds [14, 15, 16, 17]. Endo et al. 1986 also reported that the lovastatin can be produced by various other

filamentous fungi such as *Penicillium, Doratomycetes, Eupenicillium, Gymnoascus, Phoma Trichoderma* species.

Negishi et al. in 1986 screened 124 species of the genus *Monasus*, of which 17 strains reported to produce lovastatin[18]. Many studies had shown that lovastatin was even produced from *Penicillum* species [19, 20, 21].

Many *Basidomycetes* species are shown to produce higher concentrations of statin drugs. Wasser et al., in 2002 patented the various composition and production methodology of cholesterol lowering molecule from *Pleurotus sp* [22].

In the year 2010, Samiee et al., screened 110 fungal strains obtained from Persian type culture collection, where the screening was done by growing the culture in two staged fermentation process; of which only nine strains were shown to produce lovastatin [23].

A new screening technique was adopted by Vilches et al in 2005, where the rapid screening technique was used for *Candida albicans*, which showed a greater zone of inhibition for the lovastatin producing organisms [24]. This method had proved to be cost effective and less time consuming.

A screening study was being carried out for 65 marine actinomycetes strains by two stage fermentation process, out of which a single strain of actinomycetes (SS16/4) produced lovastatin[25]. In India, a study was done by Sree Devi et al., (2011) to screen the lovastatin producing strains from soil samples where the strains of *Aspergillus* were isolated from the soil sample and tested. A strain of *A.terreus* MTCC 10831 alone had proved to produce a maximum lovastatin than the other strains [26].

Studies revealed that various strains are capable of producing cholesterol lowering drugs that are available in natural resources and in soils. It also revealed that many edible fungi of higher basidomycetes are capable of producing cholesterol lowering drug; especially from *Pleurotus* sp. which is used as a SCP producer.

Production of Lovastatin Using submerged fermentation

Many researches are being carried out for the production of lovastatin in submerged fermentation process using various microbes. Mainly in submerged fermentation process, the physico-chemical parameters were optimized for the better yield of lovastatin [27]. Genetically modified organisms were also used to improve and develop the strains for lovastatin production. These process optimization and mutation studies help in the development of the strains and use minimal nutrients for the maximum yield of lovastatin.

In 1980, Mevacor started the production of lovastatin in large scale using *A.terreus* strain. In this production process, various physical parameters like temperature, pH, cell homogeneity and time, were studied. This study showed that the maximum yield can be obtained from controlled pH and slower addition of carbon source and in particular, glycerol had increased the production, five folds. However during scale- up, a major problem had occurred, where the oxygen transfer rate affects the yield and act as a key rate limiting step in the production. This has been overcome by improving the impeller and increasing the hydrodynamic thrust [28].

Osman and his group in 2011 studied the physical and chemical parameters of lovastatin production using the common strains of *A.terreus*. The studies reveled that the maximum production of lovastatin was seen when oat meal was used as a carbon source at a concentration of 20g/l, increasing this concentration further leading to the inhibition of lovastatin production. In his studies, agitation caused a negative effect in the production of lovastatin, and other physical parameters which were found to be optimum are temperature 30°C, pH of 8.5 and a time of eight days [29].

To obtain a maximum yield of lovastatin, a stastical optimization was done using a box-benken design, by Casas Lo´pez 2003; where the

carbon, nitrogen, and oxygen levels were optimized. The study showed that the carbon, nitrogen and oxygen concentration of 48 g/dm3, 0.46 g/dm3, 0.79 g/dm3 were the optimum condition for the maximum yield from *A.terreus* [30]. This model also helps in understanding the relationship between various parameters for a maximum yield.

Casas Lo´pez in 2004 also studied the effect of different carbon and nitrogen in the ratio for the production of lovastatin in *A.terreus* ATCC 20542. Parameters like lactose, glucose, fructose, yeast extract, cornsteep liquor and soyabeen meal were studied and found that lactose gave a maximum yield in a ratio of soyabean meal with yeast extract [31]. The studies also revealed that the glucose at higher concentration showed a repression effect and higher concentration of nitrogen source also affects the production of lovastatin.

A new two stage fermentation process was developed for the production of lovastatin, where the yield was increased to 315% compared to a batch fermentation process. In two staged fermentation process, nitrogen free medium was used along with many minerals and biotin as an inducer that helped in increasing the production yield at 96 hrs [32].

A.terreus MTCC 10831 was mutated using physical (UV) and chemical (Ethyl Methyl Sulphonate) method and studies were compared with the wild strains. A 32.878% increase in production was observed when mutated with UV wherein with EMS mutant strain an increase of 84.17% was recorded when compared with native strains [33].

In 1997, a repeated fed batch fermentation process was carried out for the production of lovastatin using *A.terreus* and found that 37% increase in yield was obtained when compared to batch fermentation process [34]. A similar study was done by Sitaram Kumar in 2000, where the carbon and nitrogen ratio was optimized in *A.terreus* DRCC122 strain [35] and the yield was increased to 73%.

A study was carried out to find the effect of different vegetable oil as substrate like sesame, sunflower, soyabean, corn, palm and olive oil and the tested results were found that palm oil showed a 4.5 fold increase in lovastatin production and also proved that the increase in lovastatin concentration affected its yield, which may be due to poor oxygen transfer [36].

Similarly to maximize the lovastatin production in the large scale fermentation process, the rate limiting step of oxygen transfer was studied using many oxygen carriers like n-dodecane, n-tetradecane and n-hexadecane. Exponential increase in lovastatin was found and even a better yield was seen when the oxygen carrier was added after 24 hrs of fermentation process [37].

Different models were analyzed for maximum production of lovastatin using various fungal species. This model helps in understanding the relationship between the various parameters and the optimum condition that can be used for better yield. A fuzzy model was developed for the production of lovastatin from *A.terreus* in an air lift reactor, where the pellets were used in the production. For the trail run, the dilution rate and the biomass concentration were used as an input function and also this mode helped in increasing the productivity to 1.3 folds [38].

Many studies showed that *A.terreus* is an efficient producer of lovastatin, but it also produces various other substances like geodin, sulchorine molecules; which affects the lovastatin production. The geodin molecule was produced along with the lovastatin production, which showed a similar mechanism as that of lovastatin, where they both produced at the same concentration and also they were independent of biomass[39].

Other than *A.terreus*, many other species were also reported to produce lovastatin, in which the most studied organism is the *Monascus species*. The effect of the carbon and nitrogen sources were tested for the production of lovastatin from *Monscuss pilosus*, where the maxium yield was obtained in nitrogen reduce medium and also a combination of glucose and glycerol gave the maximum yield [40].

Other carbon, nitrogen sources with many other micronutrients like salts, minerals were also affected the production of lovastatin from *Monascuss pureus*. Optimization of these parameters was done by

Placket Burman design in which both the physical and chemical properties were studied for maximum yield of lovastatin and the design also helped in understating the statistical model in which the confidence level was found to be more than 90% [41, 42].

Taguchi method was used by Chung, 2007 for optimization of lovastatin production, where he studied five factors in three levels. The production was studied both at growth phase and metabolic phase [43]. Results revealed that the production was observed in both the phases in acidic pH and also the maximum yield was seen in the growth phase.

Production of Lovastatin Using Solid State Fermentation:

Solid state fermentation is a process where a wide range of agricultural waste, polymer can be used for growing fungal species. Because of ease optimization parameters, maximum utilization of substrate and simple downstream processing steps, solid state fermentation was chosen for the growth of bacteria and fungi [44].

Biocon is the first Indian company that produced a pharmaceutical compound from fermentation. Biocon had received an appropriate approval from FDA (Food and Drug Administration) for the production of Lovastatin, which was patented in year 2001. A large scale PLA factor was used as a solid-matrix for the production of lovastatin [45]. This has some remarkable advantages of operating both in Solid and submerged fermentation process in turn reduces the downstream processing problems.

The use of solid substrate for the production of enzymes and other theraputical products would be a cost effective method. The fungal strains that produce lovastatin have the ability to grow on a wide range of solid substrates with optimum conditions. The maximum lovastatin production was seen when *A.terreus* was grown on wheatbran (982.3µg/g). It has an ability to grow and produce on various substrates like sorghum, rice bran and paddy straw, which also showed activity [46]. Mutation does not have any effect on lovastatin production, as mutated *A.terreus* showed similar yield when wheat bran was used as a substrate [47].

A.fischeri showed increased lovastatin production when blackgram husk was used as a substrate. In this process, the yield was increased by the addition of synthetic carbon sources like lactose and malt extracts [48]. Various species of *Aspergillus* proved their ability to produce lovastatin using wheat bran as a substrate. Various other species like *Monascus sp*, *Penicillium funiculosum, Pleurotus ostreatus* have also produced lovastatin using wheat bran as substrate [49,50,51]. Other than wheat bran, ricebran also showed higher yield of lovastatin using different cultures. *Aspergillus terreus* proved to produce sulochrin molecules along with lovastatin; but the production time varied between them. The maximum production of lovastatin and sulochrin was seen on day seven and day five respectively [52]. *Aspergillus parasiticus* NCIM 696 was not able to produce any other compound with rice bran as substrate for the production. A yield of 9.2 mg/gds was obtained at an optimum condition of incubation (120 h), Temperature (28oC), Moisture content (70%), Inoculum age (6 days), Inoculum volume (20% v/w), pH of the medium (6.0) [53].

Carbon and nitrogen sources have great influence on lovastatin production, which act as a rate limiting step for the growth and biomass formation. Xub, Wang Q and his team studied the influence of carbon and nitrogen sources and reported that rapid glucose metabolism resulted in an increased biomass formation, which also affected the yield of lovastatin by reducing the oxygen transfer in the culture. But an initial glucose supplementation increased the high product accumulation in the culture, while suppression of the yield occurs with the presence of organic nitrogen sources.

Monascuss sp. proven to produce lovastatin through solid state fermentation process, in which various substrates were analyzed for the production and found that rice bran gave higher yield by optimizing various substrate [54, 55]. The optimum condition can be attained efficiently by Response Surface Methodology (RSM) to assess the lovastatin production. *M. purpureus* MTCC 369 was optimized under SSF where the yield of 3.432 mg/g was obtained at an optimum inoculum level of 5 ml, pH 6.00 and a time period of 14.43 days [56]. Mutant strain of *Aspergillus terreus* KLVB28mu21 gave a maximum yield of 1110g/dry gw of lovastatin, where the SSF was carried out when wheat bran was used as a substrate at an optimum condition of pH 5.5, Temperature 30°C, moisture content of 65%, and an inoculum size of 1 X 108 spres mL-1 [57].

The maximum production is the SSF was confirmed by isolating and analyzing the DNA and RNA by corresponding blotting techniques. The results confirmed that the lova genes (lova b and lova f) were found maximum in the SSF when compared to SmF [58].

SSF is an efficient method for the production of industrially important products from diverse microbial sources. Certain limitations that are possible for the productions of industrial products through SSF are the process controlling parameters, scale up for industrial process from laboratory levels.

Applications

HMG-CoA reductase inhibition by lovastatin creates many metabolisms of isopernoids. These are vital in the control of cell growth and differentiation. Thus lovastatin shows pleiotropic effects [59]. Lovastatin had proven to reduce the coronary heart diseases (CHD) in the patients, by lowering the plasma cholesterol level. The clinical trials demonstrated that the statin not only reduce the plasma cholesterol level but also reduced the mortality in the patients [60]. Lovastatin controlled the CHD by improving the endothelial response; maintain plaque stability and prevented the thrombus formation [61]. Cholesterol formation was stopped by lovastatin, by inhibiting the HMG-CoA enzyme which is the important enzyme for the formation of mevolanate from 3-hydroxy-3-methylglutaryl coenzyme A [62]. The rate limiting step of cholesterol synthesis was blocked by covalent binding to the substrate analogue of reductase enzyme. Lovastatin reduces the LDL (low density lipoprotein) and increases the HDL (High density lipoprotein) level, and it also reduces the lession formation, yet the mechanism of action is unclear.

Alzheimer's disease

Lovastatin treatment had shown to the prevalence of Alzheimer's disease in patients [63]. The mechanism of action was not clearly known. Alzheimer's disease which was caused by the production of the neurotoxic amyloid- beta protein (AP) in the brain leads to the degradation of brain nerve cells [64]. Lovastatin showed a decrease of Alzheimer's disease level in animal cell culture; while in human trial does not give a 100% efficiency result.

Multiple Sclerosis:

Patients, who administrated with lovastatin, had shown preventing from Multiple sclerosis. The studies showed that lovastatin suppressed the Tumor Necrosis Factor (TNF α) and also revealed that there was a decreased level of inflammatory response. These responses were occurred by the up regulation of Antigen presenting cell (APCII) and the inhibition of MHCII [65].

Renal disease treatment

The down regulation of inflammatory and cytokine activity of GTPases RAS super family helps in the treatment of renal disease. Lovastatin also influenced the intercellular signaling pathway, which plays a crucial role in cell signal transduction and cell activation. This helps in the prevention of kidney damage, especially in the glomerulus nephritis associated kidney disorder. But the exact role of lovastatin is yet to be elucidated [66].

Bone Maturation

Garrett et al. (2007) studied the effect of lovastatin in the bone maturation; studies were done by injecting the nano lovastatin particles with high dose of lovastatin. The results revealed that the

high dosage stimulated high bone formation both invivo and invitro and also the injection of lovastatin at particular sites heal the femoral fractures and decreased the cortical fracture gap. These bon formations were highly seen at high dosage, where there is stimulation in the formation of murine long bones. These studies revealed that lovastatin can be administrated as nano beads, which can be used in the repair of bone fractures [67].

Anticancer

Lovastatin compounds had a great effect on cancer cells but the site of actions and the mechanism of actions are not known. Tandon et al. (2005). Studied the effectiveness of cancer cells and anti-proliferative effects were seen [68]. Glyn et al. (2008) had shown that acute transfer of lovastatin at specific sites reduced the cancer level nearly 20%- 55% [69]. A specific study was done by Xia et al. (2001) on the proliferation of cancer in human glioblastoma cell, and there was a reduction in the cancer level by inhibition of RAS farsenylation[70].

CONCLUSION

The review clearly says that various fungus have ability to produce lovastatin both by submerged and solid state fermentation process. Even though more studies were done on submerged fermentation process, solid state fermentation is an effective method for the production of lovastatin at low cost. Therefore, more studies need to be done for the effective production of lovastatin using agricultural waste as substrate, through SSF. The review also shows the wide range of medicinal properties of lovastatin in lab scale, but it seems to have more unseen application. Mechanisms of action need to be done to understand the application and used as a drug in humans for other diseases.

REFERENCE

1. Hanukoglu I. Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. J Steroid Biochem Mol Biol.1992; 43: 779–804.
2. Robert K.Murray, Daryl K. Granner, Peter A.Mayes, Victor W. Rodwell, Cholesterol synthesis, transport and excretion. Peter A.Mayes, Kathleen M.Botham. 26th ed. Mc Graw Hill, 2003, pp 219-241.
3. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. The Expert Panel. Arch. Intern. Med. 1998; 148: 36–69
4. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, Witztum JL . Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. Diabetes Care. 2008; 31: 811–22.
5. Kirby. T. J. Cataracts produced by triparanol. (MER-29). Trans. Am. Ophthalmol. Soc.1967; 65, 494–543.
6. Endo A, Kuroda M, Tanzawa K. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-562236A and ML-236B fungal metabolites having hypocholesterolemic activity. FEBS Lett. 1976; 72:323–326.
7. Chan JK, Moore RN, Nakashima TT, Vederas JC. Biosynthesis of mevinolin. Spectral assignment by double-quantum coherence NMR after high carbon-13 incorporation. J Am Chem Soc. 1983; 105:3334–3336.
8. Moore RN, Bigam G, JK, Hogg AM, Nakashima TT, Vederas JC. Biosynthesis of the hypocholesterolemic agent mevinolin by *Aspergillus terreus*. Determination of the origin of carbon, hydrogen, and oxygen atoms by 13C NMR and mass spectrometry. J Am Chem Soc. 1985;107:3694–370
9. Shiao M, Don H. Biosynthesis of mevinolin, a hypocholesterolemic fungal metabolite, in *Aspergillus terreus*. Proc Natl Sci Counc. 1987; 11:223–231
10. Endo A. Compactin (ML236B) and related compounds as potential cholesterol-lowering agents that inhibit HMG-CoA reductase. J Med Chem. 1985; 28:401–405.
11. Jonathan A.Tobert. Lovastatin and beyond: the history of the hmg-coa reductase inhibitors. Nature review. 2003; 2:517-526.
12. Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C. Mevinolin: a highly potent competitive inhibitor of hydroxymethyl glutaryl coenzyme A reductase and cholesterol lowering agent. Proc Natl Acad Sci USA. 1980;77:3957-61.
13. Greenspan MD, Yudrovitz JB. Mevinolinic acid biosynthesis by *Aspergillus terreus* and its relationship to fatty acid biosynthesis. J Bacteriol. 1985; 162:704–707.
14. Endo A. Monacolin K, a new hypocholesterolemic agent produced by Monascus species. J Antibiot (Tokyo). 1979; 32:852– 854.
15. Endo A. Monacolin K, a new hypocholesterolemic agent that specifically inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Antibiot (Tokyo). 1980; 33:334–336.
16. Endo A. Microbial phosphorylation of compactin (ML-236B) and related compounds. J. Antibiot (Tokyo). 1985b; 38:328– 332
17. Komagata D, Shimada H, Murakawa S, Endo A. Biosynthesis of monacolins: conversion of monacolin L to monacolin J by a monooxygenase of *Monascus ruber*. J Antibiot (Tokyo). 1989; 42:407–412.
18. Negishi S, Cai-Huang Z, Hasumi K, Murakawa S, Endo A. Productivity of monacolin K (mevinolin) in the genus Monascus. J Ferment Eng. 1986; 64:509–512
19. Hosobuchi M, Shiori T, Ohyama J, Arai M, Iwado S, Yoshikawa H. Production of ML-236B, and inhibitor of 3-hydroxy-3- methylglutaryl CoA reductase, by *Penicillium citrinum*: improvement of strain and culture conditions. Biosci Biotechnol Biochem. 1993; 57:1414–1419.
20. Konya A, Jekkel A, Suto J, Salat J. Optimization of compactin fermentation. J Ind Microbiol Biotechnol. 1998; 20:150– 152.
21. Bazaraa WA, Hamdy MK, Toledo R. Bioreactor for continuous synthesis of compactin by *Penicillium cyclopium*. J Ind Microbiol Biotechnol. 1998; 2:192–202.
22. Wasser SP, Reshetnikov SV Process for producing, methods and compositions of cholesterol lowering agents from higher basidiomycetes mushrooms. US Patent. 2002; 6: 372 462.
23. Siamak M. Samiee, Nasrin Moazami, Saeid Haghighi, Farzaneh Aziz Mohseni, Saeid Mirdamadi and Mohammad Reza Bakhtiari. Screening of Lovastatin Production by Filamentous Fungi. Iran Biomed J. 2003; 7: 29-33.
24. Vilches Ferro´n M.A., Casas Lo´pez J.L,. Sa´nchez Pe´rez J.A, Ferna´ndez Sevilla J.M. and Chisti Y. Rapid screening of *Aspergillus terreus* mutants for overproduction of lovastatin. World J Microb Biot. 2005; 21: 123–125.
25. Srinu M, Phani G.V. Bhusha N., Feleke moges, Srilakshmi J, Sankar G, Prabhakar T, Lakshminarayana K. Screening of Hmg Co A Reductase Inhibitor producing Marine Actinomycetes. JPRHC. 2010; 2: 66-74.
26. Sree Devi K, Venkateswara Rao J, Lakshmi Narasu M, SaiKrishna K. Isolation and screening of lovastatin producing *Aspergillus terreus* fungal strains from soil samples. IJPT. 2011; 3: 2772-2782.
27. Manzoni M, Rollini M. Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. Appl Microbiol Biotechnol. 2002; 58:555-64.
28. Buckland B, Gbewonyo K, Hallada T, Kaplan L, Masurekar P. Production of lovastatin, an inhibitor of cholesterol accumulation in humans. In: Demain AL, Somkuti GA, Hunter- Cevera JC, Rossmore HW (eds) Novel microbial products for medicine and agriculture. Elsevier, Amsterdam. 1989; 161–169.

29. Osman M.E.,. Khattab O.H,. Zaghlol G.M, Rehab. M. Abd El-Hameed. Optimization of Some Physical and Chemical Factors for Lovastatin Productivity by Local Strain of *Aspergillus terreus,* Aust J Basic and Appl Sci. 2011; 5: 718-732.
30. Casas Lo´pez JA , Sa´nchez Pe´rez JA, Ferna´ndez Sevilla JM, Acie´n Ferna´ndez FG, Molina Grima and Chisti Y. Production of lovastatin by *Aspergillus terreus*: Effects of the C:N ratio and the principal nutrients on growth and metabolite production. Enzyme Microb Tech.2003;33:270–277.
31. Casas Lo´pez JL, Sa´nchez Pe´rez JA, Ferna´ndez Sevilla JM, Acie´n Ferna´ndez FG, Molina Grima E and Chisti Y. Fermentation optimization for the production of lovastatin by *Aspergillus terreus*: use of response surface methodology. J Chem Technol Biotechnol. 2004; 79:1119–1126.
32. Elisa M Rodr´ıguez Porcel, Jose L Casas Lo´pez, Jose A Sa´nchez Pe´rez and Yusuf Chisti; Lovastatin production by *Aspergillus terreus* in a two-staged feeding operation. J Chem Technol Biotechnol. 2008; 83:1236–1243.
33. Sreedevi K, VenkateswaraRao J, Lakshmi Narasu and Fareedullah Md. Strain improvement of *Aspergillus terreus* for the enhanced production of lovastatin, a HMG-COA reductase inhibitor; J. Microbiol. Biotech. Res. 2011; 1:96-100.
34. Novak N, Gerdin S, Berovic M. Increased lovastatin formation by *Aspergillus terreus* using repeated fed-batch process. Biotechnol Lett .1997; 19:947-948.
35. Sitaram Kumar M, Swapan K. Jana, Senthil V, Shashanka V, Vijay Kumar S, Sadhukhan AK. Repeated fed-batch process for improving lovastatin production. Process Biochem.2000; 36: 363–368.
36. Pattana Sripalakit , Janya Riunkesorn and Aurasorn Saraphanchotiwitthaya. Utilisation of vegetable oils in the production of lovastatin by *Aspergillus terreus* ATCC 20542 in submerged cultivation. Maejo Int. J. Sci. Technol. 2011; 5: 231-240.
37. Long-Shan T. Lai, Tai-Her Tsai, and Te Chi Wang. Application of Oxygen Vectors to *Aspergillus terreus* Cultivation. J Biosci Bioeng. 2002; 94:453-459.
38. Kamakshi Gupta, Bodhisatta Maiti, P K Mishra, Pradeep Srivastava. Fuzzy rule-based prediction of lovastatin productivity in continuous mode using pellets of Aspergillus terreus in an airlift reactor. J Biochem Tech. 2009; 2:138-143
39. Marcin Bizukojc, Stanislaw Ledakowicz. Simultaneous biosynthesis of (+)-geodin by a lovastatin-producing fungus *Aspergillus terreus.* J Biotechnol.2007; 132:453–460.
40. Tsuyoshi Miyake, Kumiko Uchitomi, Ming-Yong Zhang; Nobuyuki Nozaki, Hiroyuki Sammotoand Kenji INAGAKI. Effects of the Principal Nutrients on Lovastatin Production by *Monascus pilosus*. Biosci. Biotechnol. Biochem.2006;70: 1154–1159.
41. Sadik Ali Sayyad & Bibhu Prasad Panda & Saleem Javed & Mohd Ali. Optimization of nutrient parameters for lovastatin production by *Monascus purpureus* MTCC 369 under submerged fermentation using response surface methodology. Appl Microbiol Biotechnol. 2007; 73:1054–1058
42. Subhagar Seraman, Aravindan Rajendran, Viruthagiri Thangavelu. Statistical optimization of anticholesterolemic drug lovastatin production by the red mold *Monascus purpureus*. Food Bioprod Process. 2010; 88:266–276.
43. Chung C.C, Chen H.H. and Hsieh. P.C. Application of the taguchi method to optimize monascus spp. Culture. J Food Process Eng.2007; 30: 241–254.
44. Pandey A, Soccol CR, Rodrigoez-Leon and Nigam P. Solid state fermentation in biotechnology- Fundamentals and Applications Ist ed. New Delhi India: Asiatech Publications Inc 2001
45. Praveen, Savitha, J ; Solid State Fermentation: An Effective Method for Lovastatin Production by Fungi – A Mini Review. The Open Tropical Medicine Journal. 2012; 5:1-5.
46. Jaivel N and Marimuthu P. Optimization of lovastatin production in solid state fermentation by *Aspergillus terreus*. Int J Eng Sci Technol.2010; 2: 2730-2733.
47. Ruchir C. Pansuriya; Rekha S. Singhal; response surface methodology for optimization of production of lovastatin by solid state fermentation. Braz J Microbiol. 2010; 41: 164-172.
48. Pallem akya, Parvatham Madhu Latha , Manipati Srikanth; Solid state fermentation for the production of lovastatin by *Aspergillus fischeri*. Res J Pharm Sci Biotech. 2011;1:9-13.
49. Sri Rami Reddy D, Prasanna Latha D, Hema Latha KPJ. Production of Lovastatin by Solid State Fermentation by *Penicillium Funiculosum* NCIM 1174. Drug Invention Today. 2011; 3: 75- 77.
50. Valera H.R., Gomes J., Lakshmi S., Gurujara Ra., Suryanarayan S., Kumar D. Lovastatin production by solid state fermentation using *Aspergillus flavipes*. Enz. Microbiol. Technol. 2005; 37: 521-526.
51. Lakshmanan D and Radha K.V; An effective quantitative estimation of lovastatin from *Pleurotus ostreatus* using UV and HPLC. Int J Pharm Pharm Sci.2012; 4: 462-464.
52. Rizna Triana Dewi, Nina Artanti, Hani Mulyani, Puspa Dewi Narrij Lotulung, and Minarti; Production of lovastatin and sulochrin by *Aspergillus terreus* using solid state fermentation; Makara, Teknologi. 2011;15:1-4.
53. Geeta Aparna D and Sri Rami Reddy D. Production of lovastatin by *Aspergillus parasiticus* NCIM 696 using rice bran under solid state fermentation. J. Chem. Bio. Phy. Sci. Sec. B. 2011; 2: 284-291.
54. Seraman Subhagar Rajendran Aravindan Thangavelu Viruthagir. Response surface optimization of mixed substrate solid state fermentation for the production of lovastatin by *Monascus purpureus*. Eng. Life Sci. 2009; 9: 303–310.
55. Bibhu Prasad Panda, Saleem Javed and Mohamed Ali; Engineering rice based medium for production of lovastatin with Monascus Species. Czech J. Food Sci. 2009; 27:352–360.
56. Panda BP, Javed S, Ali M. Statistical analysis and validation of process parameters influencing lovastatin production by *Monascus purpureus* MTCC 369 under solid state fermentation. Biotechnol Bioprocess Eng. 2009; 14: 123-7.
57. Prabhakar M. Lingappa K. Vivek babu. Amena S. Vishalakshi N. AND Mahesh D. Characterization of physical factors for optimum lovastatin production by *Aspergillus terreus* KLVB28mu21 under solid state fermentation. JRAAS. 2012; 27:01-05.
58. Barrios G, Barrios JG, Covarrubias AA, Arroyo AG. Lovastatin biosynthetic genes are expressed differentially in solid state and in liquid submerged fermentation. Appl Microbiol Biotechnol 2008; 79:179-86.
59. Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS. ACC/ AHA 2002 guideline update for the management of patients with chronic stable angina – summary article: A report of the American College of Cardiology/ American Heart Association Task Force on practice guidelines. J Am Coll Cardiol. 2003;41:159-68.
60. Seenivasan, Subhagar S., Aravindan R. And Viruthagir. T. Microbial Production and Biomedical Applications of Lovastatin. Indian J Pharm Sci. 2008;70:701-709.
61. Pickin DM, McCabe CJ, Ramsay LE, Payne N, Haq IU, Yeo WW. Cost effectiveness of HMG-CoA reductase inhibitor (statin) treatment related to the risk of coronary heart disease and cost of drug treatment. Heart. 1999;82:325-32.
62. Alberts AW, Chen J, Kuron G, Hunt V, Huff J, 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. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and cholesterol lowering agent. Proc Natl Acad Sci USA.1980; 77:3957–3961.

63. Eckert GP, Wood WG, Muller WE. Statins: Drugs for Alzheimer's disease?. J Neural Transm. 2005; 112: 1057-71.
64. Ohm TG, Meske V. Cholesterol, statins and tau. Acta Neurol Scand. 2006;114:93-101.
65. Zamvil SS, Steinman L. Cholesterol-lowering statins possess anti- inflammatory activity that might be useful for treatment of MS. Neurology. 2002; 59: 970-1.
66. Buemi M, Senator M, Corica f. Satins and progressive renal disease. Med Res Rev. 2002; 22: 76-84.
67. Garrett IR, Gutierrez GE, Rossini G, Nyman J, McCluskey B, Flores A, Mundy GR. Locally delivered lovastatin nanoparticles enhance fracture healing in rats. J. Ortho. Res. 2007; 25: 1351-1357.
68. Tandon V, Bano G, Khajuria V, Parihar A, Gupta S. Pleiotropic effects of statins. Ind J Pharmacol. 2005; 37: 77-85.
69. Glynn SA, O'Sullivan D, Eustace AJ, Clynes M, O'Donovan N. The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, simvastatin, lovastatin and mevastatin inhibit proliferation and in- vasion of melanoma cells. BMC Cancer. 2008; 16: 8-9.
70. Xia Z, tan MM, Wing WW, Dimitroulakos J, Minden MD, Penn LZ. Blocking protein geranylgeranylazation is essential for lovastatin induced apoptosis of human acute myeloid leukemia cells. Leukem.2001; 15: 1398-407.