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 mevalonate from Acetyl CoA. Inhibition is done by statin drug, which is produced by many fungi through polyketide pathway. Many fungi produce lovastatin, while other statins like rosvastatin, 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 understanding the inhibition of HMG-CoA reductase and various medicinal properties of lovastatin.

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

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-CoA in a complex 37 steps process, where the rate limiting step was the conversion of HMG-CoA to mevalonate using HMG-CoA reductase [2]. These cholesterol 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 artherosclerosis, 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 2010 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. Triparanol is the first anti-cholesterol drug discovered in the year 1958. It reduces the cholesterol level in the blood by inhibiting deesterol kinase by controlling the formation of cholesterol. The drug was banned and withdrawn from market, due to its severe side effects [5]. Later a new drug MIL2368 (Compactin) was discovered by Masuo Kuroda and Akira Enido [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 cholesterol 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 Aterreus 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 rosvastatin, 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. Aterreus is a strain that was used in the production of lovastatin, which was widely studied and an immense 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
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 one single strain of actinomycetes (S116/4) 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 researchers are being carried out for the production of lovastatin in submerged fermentation process using various microbes. Mainly in submerged fermentation process, the physicochemical 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 revealed 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 statistical optimization was done using a box-benken design, by Casas Lopez in 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 Lopez 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, corn steep liquor and soyabean 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 31.5% 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, soya bean, 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 ofLovastatin 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 trial 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, sulchrone 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 *Monascus pilosus*, where the maximum 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 Monascus purpureus. 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 understanding 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 metabolism 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 therapeutical 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 wheat bran (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, rice bran 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 (280°C), Moisture content (70%), Inoculum age (6 days), Inoculum volume (20% v/v), 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.

Monascus 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 1110μg/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 spores 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 isoprenoids. 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 mevalonate 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 lesion 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 the 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 bone formation 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


