Solid State Fermentation: An Effective Method for Lovastatin Production by Fungi – A Mini Review

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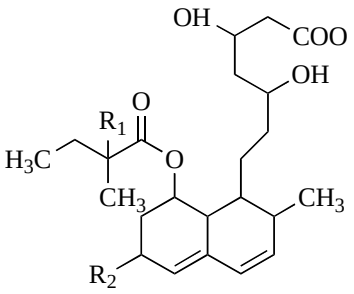
Abstract: Lovastatin, an anti-cholesterol drug, is a potent inhibitor of the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase) that catalyses conversion of HMG CoA to mevalonate involved in cholesterol biosynthesis. Lovastatin does not only find a role as anti-cholesterol agent but also plays a key role as an anti-inflammatory agent, cancer cell apoptosis, renal function restoration, treatment for bone disorders etc. Production of statins has been undertaken by Submerged Fermentation (SmF) and Solid State Fermentation (SSF) by using fungi. In view of the advantages of SSF over SmF, the focus has shifted to production by SSF in recent years. The use of various agro based wastes as substrates has been proven. With recent developments in molecular biology and genome studies it has been able to decode genetic aspects of lovastatin gene expression levels. This review gives an insight into works reported on lovastatin production by SSF, various substrates employed, mutation studies, optimisation parameters, molecular studies and medical application of lovastatin.

Keywords: Fermentation, fungi, HMG CoA, lovastatin, pleiotropic, SSF.

INTRODUCTION

The World Health Organisation reported that cardiovascular diseases (CVD) claimed 17.3 million lives in 2008 and predicts that an estimated 23.6 million people will die of CVD by the year 2030. The lower and middle income countries constituted 80% of the mortalities [1]. Today, the average age in which a person may suffer a heart attack has come down drastically almost to range between 30-40 years with hypercholesterolemia being one of the primal causes. The treatment for hypercholesterolemia focuses on decreasing the Low Density Lipoprotein (LDL) cholesterol and is best achieved by medications when diet and exercise are insufficient. Fungi produce a wide variety of biologically active compounds, a large proportion of which are produced by the polyketide biosynthetic pathway. Fungal polyketides comprise a very large and structurally very diverse group and many display important biological activities such as antibiotic and other related pharmacological properties [2]. Among the fungal metabolites, statins (anti-cholesterol compounds) are considered as the most important class produced by the polyketide pathway. Statins comprise of Compactin Lovastatin, Pravastatin, Simvastatin, Rosuvastatin, Atorvastatin and Fluvastatin. Compactin and lovastatin are of biological origin whereas simvastatin, pravastatin, rosuvcastatin etc are chemical modifications of compactin and lovastatin [3]. The biosynthetic pathway involved in statin production starts from acetate units linked to each other in head to- tail fashion to form polyketide chains. Lovastatin prevents formation of mevalonate from HMG Co-A [4, 5] 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 very lipophilic while the acid forms are hydrophilic (e.g. atorvastatin, fluvastatin, pravastatin [6]. The global ruling rate of statins is $400-$1200 per kilogram with a growth rate of 30% per annum (The Economic Times, October 13, 2003.). Lovastatin and other natural and synthetic derivatives constitute the statin group that has caught the eye of numerous pharmaceutical companies.

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 [3]. In addition even the Basidiomycetes mushrooms have recently reported to be used. Pleurotus sp and its related strains produce higher concentrations of lovastatin. A marine actinomycete has also been reported to produce lovastatin [7]. This review gives an insight into lovastatin production by SSF approach in the last 5-6 years and the possible multiple (pleiotropic effects) applications of lovastatin in medical field.



Open hydroxyl Form of lovastastin

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Table 1. Lovastatin Production by SmF

S. No.	Microorganism	Lovastatin Yield (mg/L)	References
1	Aspergillus terreus	0.40	Szakacs *et al.,* 1998 [32]
2	Aspergillus terreus	2200	Kumar *et al.,* 2000 [33]
3	Aspergillus terreus	55	Samiee *et al.,* 2003 [34]
4	Monascus pilosus	725	Miyake *et al.,* 2006 [35]
5	Monascus purpureus	0.318	Sayyad *et al.,* 2007 [36]
6	Monascus purpureus	737	Ahmed *et al.,* 2009 [37]

History

In the 1950's a US based company, Wm. S. Merrell Co., identified a compound with anti-cholesterol property and named it as Triparanol (MER/ 29) which blocked the conversion of desmosterol to cholesterol, the final stage in cholesterol biosynthesis. Later it was reported that triparanol is ineffective drug and associated with lens cataracts, hair loss in rats and dogs, also higher doses causing blindness in rats. 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 what they were seeking for. The metabolite from *Penicillium citrinum* was christened as ML236B (referred to as compactin), an anti-cholesterol agent. With several tests the carcinogenic effects of compactin came to light as they noted lymphomas in dogs treated with higher doses of compactin. This led to company to abruptly halting clinical trials. Later, Merck, a competitor had initiated works on discovering anti-cholesterol drug and reported the production of Mevinolin (Lovastatin) from *Aspergillus terreus*. Later, it was discovered that in fact what they called 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 FDA in 1987 to release Mevinolin into the market, thus, starting the statins era. In 1979, Endo reported that *Monascus sp* also produced reductase inhibitors and obtained a patent for the same [8, 9].

Pleiotropic Effects of Lovastatin

The clinical, epidemiological and pathological applications of lovastatin have been well documented. It finds its 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 [10, 11].

Alzheimers Disease (AD): AD is associated with formation of amyloid plaques in brain due to production of neurotoxic amyloid β protein (produced by alternate processing of amyloid precursor protein). Animal and cell culture experiments reported decreased prevalence of AD using lovastatin. Human trials did not succeed 100% and more work needs to be warranted in the same area to attribute exact role of lovastatin [12].

Multiple Sclerosis (MS): Lovastatin supressed production Tumor Necreosis Factor- α (TNF-α) by Interferon-α (IFN-α). This resulted in decreased level of inflammatory response and protecting the host cells by cellular damage. 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. This indicates immune-modulatory role of lovastatin [6].

Bone Disorders: prolonged infusions or large doses of lovastatin stimulate bone formation both *in-vivo* and *in-vitro* as noted in murine models. Pre-clinical studies report that nano particle delivery of lovastatin may fasten human bone fractures [13]. This beneficial effect may also be used in treatment of osteoporosis [4].

Renal Protection: kidney damage associated with glomerulonephritis may be retarded by lovastatin treatment. This may be due to down regulation of inflammatory cytokines and activity of GTPases Ras superfamily. The exact role of statins is yet to be elucidated [14].

Cancer: Lovastatin is found to induce apoptotic response in human acute myeloid leukemia (AML) cells. 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. The mechanism underlying lovastatin-induced apoptosis of malignant cells remains unclear [15, 16]. Observations have found large reductions in the risk (20–55%) of site-specific cancers (colorectal, breast, prostate, lung, and pancreatic) with the use of statin therapy [16]. Inhibition of Ras farsenylation is associated with reduction and proliferation of cancer in human glioblastoma cells. Further studies need to be carried out to exactly predict if satins can be used as anti-cancer drug as some trials have failed to reproduce results [4].

Rheumatoid Arthritis (RA): Human trials using lovastatin, may help to decrease problems associated with RA by immunomodulation and modified endothelial function [4].

A recent study reports the role of statins as an immune-modulator in treatment of vitiligo and its beneficial part in graft transplant is being investigated. With its vibrant application statins in future will play a vital role in medical and pharmaceutical field [10, 11].

Solid Substrate Fermentation for Lovastatin Production

Lovastatin production and optimisation of fermentation parameters has been of great interest since its discovery. Many efforts and trials have been performed to increase the titre. Initially, all production processes were carried in Submerged Fermentations (SmF) by varying physico-nutritional parameters. The submerged processes have not but yielded constant results and higher yield (Table **1**) and hence a shift

Table 2. Lovastatin Production by SSF

S. No.	Substrate	Microorganism	Lovastatin Yield	References
1	Black gram husk	*Aspergillus fischeri*	12.63 mg/g	Chanakya *et al.*, 2011 [23]
2	Black gram husk Green gram husk Orange peel Wheat bran	*P funiculosum* NCIM 1174	4.8 mg/g 4.6 mg/g 3.4 mg/g 5.3 mg/g	Reddy *et al.*, 2011 [19]
3	Wheat bran	*Aspergillus terreus*	983.3 µg/g	Jaivel and Marimuthu, 2010 [22]
4	Rice based medium	*Monascus purpureus*	3.42 mg/g	Panda *et al.*, 2009 [27]
5	Wheat bran	*Aspergillus terreus* KLVB28mu21	1110g/gm	Prabhakar *et al.*, 2011 [18]

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. Many bacteria and fungi have been utilised for production of industrially important products by SSF [17].

Lovastatin production also has been carried by SSF approaches with promising results. The results were far surprising that expected with high titre of 1110g/gm dry weight of substrate [18, 19], thus portraying immense potentials of SSF (Table **2**). Numerous data is present regarding lovastatin production by SmF and optimisation parameters reported [5, 20, 21].

The production of lovastatin by solid state fermentation (SSF) has gained popularity as it involves lower media cost, stability of the product, increased yield [4, 22] and increased porosity [18]. The higher yield associated with SSF is primarily due to increased mycelial density provided with an optimum moisture range between 60%-70% [18]. The substrates used in fermentation include wheat bran, rice bran, non-glutinous rice, orange peels, grain husks [19, 22, 23]. Of all the substrates used, wheat bran has topped as the best one with a yield of 3273.4 µg/g [24] and a yield range of 806 mg/L to 982.3 mg/L [22]. An increase or decrease in moisture content is reported to affect the oxygen and water balance [24] and thereby decreasing lovastatin yield. 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* [18]. Rice bran was of good choice specially when supplemented with carbon & nitrogen source (as rice is poor in nitrogen source) and better compared to rice husk [25]. Low protein content may be dealt by addition of peptone in substrate. Rice based un-supplemented medium yields 1.703 mg/g lovastatin using *M. purpureus* MTCC 369. Rice supplemented with soya bean powder, sucrose and yeast extract inoculated with *M ruber* also resulted in high titre [26].

Lactose is a slowly metabolized product compared to glucose. Rapid glucose metabolism results in formation of alcohol, aids in filamentous growth thus resulting in increased medium viscosity and decreased oxygen diffusion therefore affecting yield. Initial glucose supplementation in bran helped *A terreus* in higher product accumulation and the presence of organic nitrogen source reportedly suppressed lovastatin yield, but too low concentration affects biomass formation too [26]. Inorganic nitrogen sources are preferred as they aid in higher titre. Selection of glucose (repressive) along with non-repressive carbon source in combination yields increased lovastatin titre in *M pilosus* [11]. Glucose and maltose are the best carbon sources (444 mg/L). Glucose is said to exert repressive action but a combination of glucose, glycerol and peptone in medium is best for *M pilosus* to produce lovastatin. Dextrose, KH_2PO_4 and $FeSO_4$ do not aid in much lovastatin production when compared to NH_4Cl, $MgSO_4$ and NaCl when present in medium inoculated with *M purpureus* 369 [27]. Various physico-nutritional parameters that govern lovastatin production have been well documented [5].

Presence of one amino acid is mandatory in the growth media. Riboflavin, Pyridoxine and Calcium phosphate 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 of180 µg/ml [5]. It is necessary to maintain a striking balance between carbon and nitrogen source for obtaining desirable lovastatin production as they regulate the biomass and metabolite production [21].

Incorporation of various supplements to the growth medium has been studied. Tween-80 addition increases the yield whereas $ZnSO_4$ had no effect on yield, but $MgSO_4$ decreased yield by 4.11% [28]. Addition of acetic acid 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 permeability [26]. Butyrolactone and Dodecane (2.5%) increased the yield by 3-4 fold [5]. Sodium acetate supplementation triggers better yield by acting as a precursor for statin synthesis [21].

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 [18, 19, 24]. 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 is incorporated into solid substrate [24]. Glucose, lactose and sucrose decreased the yield of lovastatin to 2101±51, 2534±29 and 2435±38 µg g^{-1} Dried Fermented Matter) respectively, contradictorily a higher yield was recorded when sweet sorghum syrup is incorporated with nutrient solution [22].

Mutation by Ethyl Methyl Sulphonate (EMS) and UV of *Aspergillus terreus* KLV28mu21 recorded higher yield [18]. *Aspergillus terreus* isolated from contaminated oyster mush-

room bed [22] subjected to EMS and UV mutation (strains JPM-EMS2and JPM-UV2) produced lovastatin in appreciable quantity (948.50mg/L and 1553.02 mg/L) [29].

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. Genetically engineered *A terreus* could synthesise 2,2 dimethyl butyrate (side chain of simvastatin) and produce simvastin directly rather than lovastatin [6]. 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. The expression of gldB (NADP dependent glycerol dehydrogenase), gene for osmotolerance, in SSF indicated the role of osmotic genes in *A nidulans* when inert substrate poly-urethane foam is used [30].

Response Surface Methodology (RSM) approach has been well adopted to assess lovastatin yield and determine the optimum fermentation parameters. A temperature of 29.46^{O}C, fermentation time of 14.43 days with an initial inoculum level of 5 ml at pH 6.00 yields 3.432 mg/g [31] 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 and at pH range between 5-6 and for *Aspergillus fischerii* relative humidity of 60%, pH 5, temperature 30^{0}C with lactose and malt extract as the optimum is best suited [23].

SSF hold true potential for production of many industrially important products. The limitations that may be encountered in SSF are in controlling of process parameters and scale up from laboratory to industrial level [38].

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.

CONFLICT OF INTEREST

None declared.

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None declared.

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