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Production of Statins by Fungal Fermentation

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ABSTRACT

Lovastatin serves as potent inhibitor of rate limiting enzyme in cholesterol biosynthesis which is 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase. In this way it lowers cholesterol levels in plasma effectively with nearly no side effects. Mechansim of action for lovastatin is structural similarity between acid form and HMG-CoA intermediates. Fungi are used industrially to obtain a variety of products, from low value bulk chemicals to high value drugs like, immunosuppressants, antibiotics, alkaloids and statins. Lovastatin and compact in are natural statins produced as secondary metabolites by predominantly Aspergillus and Penicillium species, following a polyketide pathway. Lovastatin was one of the first cholesterol-lowering drugs. Many statins are now chemically synthesized but lovastatin is still required to produce simvastatin. Apart from reducing blood cholesterol levels simvastatin causes pleotropic effects and has potential to treat various kinds of disorders including neurodegenerative disease and cancer.

Keywords : Statins, Fungal fermentation, Lovastatin, Simvastatin, Neurodegenerative disease

I. INTRODUCTION

Statins are drugs prescribed to reduce serum cholesterol levels. The first statin to be approved by the FDA, lovastatin, is produced as a result of fermentation of Aspergillus terreus[1]. Statins act by inhibiting HMG-CoA reductase (HMGCR) through competitive inhibition. This blocks the activity of HMGCR, the rate limiting enzyme in the synthesis of cholesterol. Natural statins, including lovastatin and mevastatin (commonly known as compactin) are produced by direct fungal fermentation. Semi-synthetic statins, simvastatin and pravastatin, are synthesised by the stereoselective hydroxylation of natural statins. Chemically synthesized statins include atorvastatin, rosuvastatin, fluvastatin and pitavastatin[2].

Discovery of statins

Compactin, discovered by Akira Endo in 1973 as a structural analogue for theHMG-CoAsubstrate, wasproduced by Penicillium citrinum. Lovastatin, previously known as mevinolin K and mevinolin, was produced from cultures of Monascus ruber and Aspergillus terreus respectively. It was the first statin to be approved by the FDA in 19874. Pravastatin, the semi-synthetic derivative of compactin was commercialised in 1989. Simvastatin remains a commonly prescribed statin.

Fungal fermentation and statin production

Statins are produced as a secondary metabolite from a polyketide pathway. This pathway is regulated by polyketide synthase genes such as Lov B, lovF and LovD, that are responsible for the transcription regulation and production of these secondary metabolites. Statins are produced as a secondary metabolite during stress of the fungi. Acetyl Co-A acts as precursor molecule that plays an important role in bridging the primary metabolism with the secondary

metabolism leading to production of various secondary metabolites such as terpenes and polyketides including statins All fungi producing lovastatin or compactin utilise the pathway shown in Figure Lovastatin is commercially produced by fermentation of A. terreus and simvastatin is produced by further chemical treatment of lovastatin usually involving direct alkylation. Compactin is not as effective in inhibiting HMGCR as lovastatin, however, a semisynthetic derivative of compactin, pravastatin, is highly effective in lowering blood cholesterol levels Different strategies have been adopted for the efficient and economic scale up of these metabolites, such as media optimisation, using cheap raw substrates, mutagenesis and bioreactor optimisation[3-4].

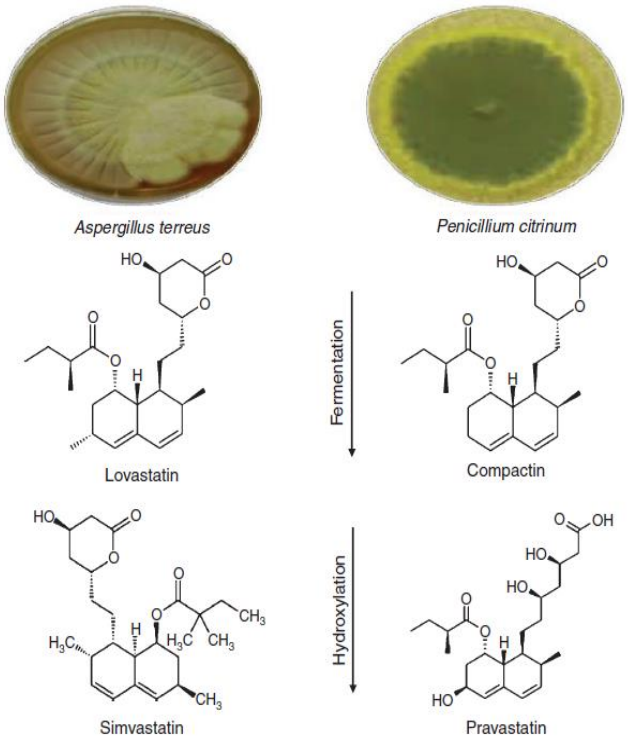

Figure 1. Production of natural and semi-synthetic statins by fungi

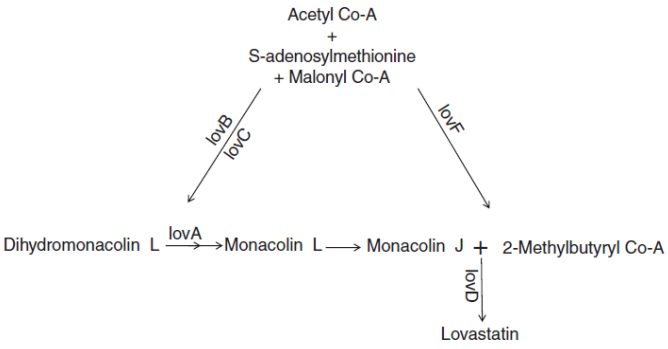

Figure 2. Pathway for the production of lovastatin in Aspergillus terreus

Unlike simvastatin where conversion of lovastatin to simvastatin is a chemical reaction, the reaction for pravastatin synthesis is biotransformation. Lovastatin can be directly methylated or deacylated for the synthesis of simvastatin. This involves a single step fermentation process and then direct chemical conversion. Pravastatin production involves the hydroxylation of compactin produced by P. citrinum by the biotransformation using the bacterium Streptomyces carbophilus. This organism produces cytochrome P450 enzyme that is responsible for the hydroxylation of compactin. This dual step fermentation is economically not feasible. Recent studies have reported the production of pravastatin in a single fermentative step. Enzymes responsible for thehydroxylation are genetically incorporated in the penicillin-producing fungus Penicillium chrysogenum. This results in efficient production of pravastatin at industrial scale[5]. Different fermentation techniques including solid state fermentation (SSF) and submerged fermentation (SmF) can be used for statin production. Large scale commercial production utilises submerged batch fermentation. There is a controlled aeration and agitation in a bioreactor during SmF, which increases the oxygen mass transfer and constant distribution of nutrients to fungal mycelia, resulting in increased production of statins. Some studies have also reported fed-batch fermentation that were carriedout in a bioreactor

with a capacity of 1000 L. Repeated fed batch processes can also improve the productivity of desired metabolites. In our studies a new species of A. terreus, MS-7, was isolated from agricultural soils. Its identity was confirmed by ITS sequence analysis and it was found to be the potent producer of lovastatin as determined by analytical HPLC. Fermentation on modified soybean meal media resulted in more lovastatin produced using SSF (13.9 mg/g) in comparison to SmF (10.3 mg/g). As lovastatin produced by the fungus is inhibitory, production by SSF might result in minimal contact of mycelia with the lovastatin underneath, enhancing the productivity. Another factor that can result in greater production is that SSF promotes more mycelial growth as compared to the SmF[6-7].

History of Lovastatin

The first ever statin was obtained from fungus, *Penicillium citrinum*, by Japanese microbiologist Akira endo in 1970.He was screening fungus for finding antimicrobial agents and that led to discovery of first statin named mevastatin[8]. In 1976, pharmaceutical company Merck and co showed keen interest in endo discovery and they produced another important statin.It was produced from another fungus, *Aspergillus terreus,* and known as lovastatin. Merck proceeding further in their work produced semisynthetic derivative of lovastatin, simvastatin, which is second leading statin in market after lovastatin. Merck and endo's dicoveries led to draw other pharmaceutical companies towards the production of synthetic statins as well[9-10]. That resulted in development of first synthetic statin, fluvastatin(Sandoz AG lescol) followed by atrovastatin with trade name Lipitor that later became the best selling drug[11].

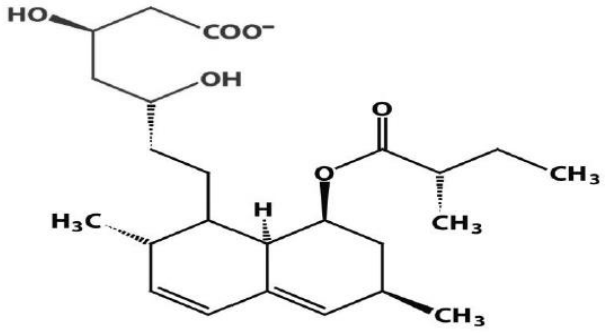

Figure 3. Structure of lovastatin

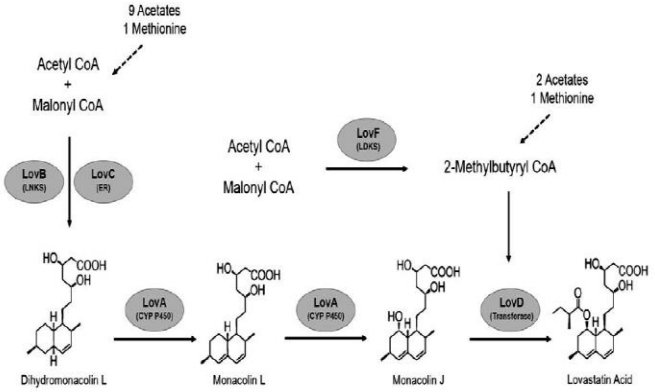

Figure 4. Genetic cluster of lovastatin

Endo and co researchers also worked further on statins and they independantly produced same product from another fungus called *Monascus ruber* but this strain could not be used for production of lovastatin on industrial scale as it produces product in low amount[12]. Lovastatin was tested in animals and then in healthy volunteers, it extraordinarily reduced blood cholesterol levels in healthy volunteers and showed no obvious side effects. Hence, lovastatin became first statin to be approved by FDA USA in 1987 after successful clinical trials with market name Mevacor. Dr Endo was awarded Japan prize in 2006 and the Lasker foundation awarded him with Clinical and Medical Research Award for his tremendous contribution towards the discovery of statins[13-15].

Mechanism of action

Lovastatin and related compounds are synthesized as predrugs that are combination of β-hydroxyacid acid form and lactone ring. This lactone ring is then transformed into β-hydroxyacid form in vivo. The

hypocholesterolemic effect of statins is carried out by a mechanism which is inhibition of HMG-CoA reductase by competitive mechanism[16-18]. This inhibition occurs due to same structures of HMG-CoA and β-hydroxyacid form of statins. The affinity of statins for reductase is several times greater than affinity of HMG-CoA intermediate. The statin occupy active site of enzyme thus blocking site for access by substrate of enzyme.The binding of statin with enzyme is due to van der walls interactions between enzyme and its inhibitor. Due to this competitive inhibition of reductase,conversion of HMG-CoA to mevalonate does not occur which is essential building block for cholesterol biosynthesis.This results in lowering of cholesterol level by inhibiting its synthesis[19].

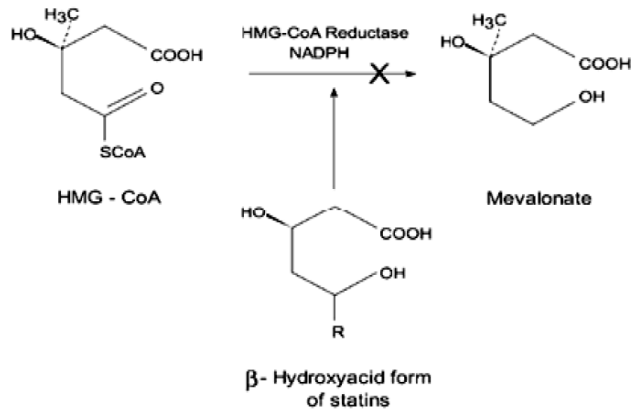

Figure 5. Mechanism of action

Application of statins

The mevalonate pathway is not only responsible for the synthesis of cholesterol but also for the synthesis of other non-sterol isoprenoids that are involved in protein prenylation such as binding and regulation of target proteins. Statins may decrease protein prenylation, a key step during a cell growth and signalling pathway. Statins can be used in combination with cancer drugs to treat cancer. Statins also reduce hepatic cholesterols leading to reduced gallstone formation and reduced platelet aggregation. Recent studies have reported the role of statins in cognitive impairment after sepsis by reversing the microvascular dysfunction and reducing neuroinflammation. Simvastatin has been found to reduce the incidence of neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease[20-22].

II. CONCLUSION

Statin and other natural as well as synthetic statins are considered as wonder drugs for treatment of hypercholesterolemia. Statins are produced by many fungal sources mainly from *Aspergillus terreus* on industrial scale. Lovastatin lowers blood cholesterol levels dramatically by inhibition of hydroxymethyl glutaryl-coenzyme A (HMG-CoA) reductase which is enzyme required for catalyzing rate limiting step for de novo cholesterol synthesis. This competitive inhibition is due to structural similarity between HMG-CoA and acid form of lovastatin. Lovastatin possess a hydroxyl hexahydro naphthalene ring which is polyketide chain to which different side chains are attached. Lovastatin synthesis starts from linkage of acetate units in head to tail fashion establishing main polyketide chain. First intermediate formed during synthesis is monacolin L which is then turned into monacolin J by hydroxylation which in turn gets converted into lovastatin. Molecular studies revealed that genetic cluster involved in biosynthetic pathway includes genes LovB, LovC, LovA, LovD, LovF and Lov H. Industrially lovastatin is produced by liquid submerged fermentation but now a day's use of solid state fermentation is gaining importance. Lovastatin serves as drug of choice for cardiovascular diseases like peripheral vascular disease, atherosclerotic plaque, peripheral arterial disease, cerebro vascular disease, sepsis and ischemic heart disease. It also shows many other biological effects beyond just lowering cholesterol level that include its effects on bone maturation, renal disorders treatment, anticancer and metabolic syndrome.

III. REFERENCES

[1]. Shiao M, Don H. Biosynthesis of mevinolin, a hypocholesterolemic fungal metabolite, in Aspergillus terreus. Proc Nat Sci Counc, 1987; 11: 223-31.

[2]. Greenspan MD, Yudkovitz JB. Mevinolinic acid biosynthesis by Aspergillus-terreus and its relationship to fatty-acid biosynthesis. J Bacteriol, 1985; 162(2): 704-7.

[3]. Subazini TK, Kumar GR. Characterization of Lovastatin biosynthetic cluster proteins in Aspergillus terreus strain ATCC 20542. Bio Information, 2011; 6: 250-4.

[4]. 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, 1989; 42(2): 407-12.

[5]. Chan JK, Moore RN, Nalashima TT, Vederas JC. Biosynthesis of mevinolin. Spectral assignment by double-quantum coherence NMR after high carbon-13 incorporation. J Am Chem Soc, 1983; 105: 3334-6.

[6]. Kennedy J, Auclair K, Kendrew SG, Park C, Vederas JC, Hutchinson CR. Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis. Am Assoc Adv Sci, 1999; 284(5418): 1368-72.

[7]. Ma SM, Tang Y. Biochemical characterization of the minimal polyketide synthase domains in the lovastatin nonaketide synthase LovB. FEBS J, 2007; 274(11): 2854-64.

[8]. Hendrickson L, Davis CR, Roach C, Nguyen DK, Aldrich T, McAda PC, et al. Lovastatin biosynthesis in Aspergillus terreus — characterization of blocked mutants, enzyme activities and a multifunctional polyketide synthase gene. Chem Biol, 1999; 6(7): 429-39.

[9]. Sutherland A, Auclair K, Vederas JC. Recent advances in the biosynthetic studies of lovastatin. Curr Opin Drug Discov Dev, 2001; 4: 229-36.

[10]. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science, 2001; 292(5519): 1160-4.

[11]. 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, Rossmoore HW (eds.). Novel microbial products for medicine and agriculture, New York; USA: 1989, p. 161-9.

[12]. Lai LS, Hung CS, Lo CC. Effects of lactose and glucose on production of itaconic acid and lovastatin by Aspergillus terreus ATCC 20542. J Biosci Bioeng, 2007; 104(1): 9-13.

[13]. Casas-López JL, Sánchez-Pérez JA, Fernández-Sevilla JM, Acién- Fernández FG, Molina-Grima E, 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 Technol, 2003; 33: 270-7.

[14]. Osman ME, Khattab OH, Zaghlol GM, El-Hameed RMM. Optimization of some physical and chemical factors for lovastatin productivity by local strain of Aspergillus terreus. Aust J Basic Appl Sci, 2011; 5(6): 718-32.

[15]. 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.

[16]. Javiel N, Marimuthu P. Isolation and screening of lovastatin producing microorganisms. Int J Eng Sci Technol, 2010; 2(7): 2607-11.

[17]. Syed, M.B, Rajasimman M. Fermentative production and optimization of mevastatin in submerged fermentation using Aspergillus terreus. Biotechnol Rep, 2015; 6: 124-8.

[18]. Jahromi MF, Liang JB, Ho YW, Mohamad R, Goh YM, Shokryazdan P. Lovastatin production

by Aspergillus terreus using agro-biomass as substrate in solid state fermentation. J Biomed Biotechnol, 2012; 2012: 1-11.

[19]. Gulyamova T, Ruzieva D, Masmetova S, Sattarova R, Lobanova K, Abdulmyanova L, Rasulova G. Lovastatin production by Aspergillus terreus in solid state and submerged fermentations. Int J Eng Sci Technol, 2013; 5: 19-24.

[20]. Pansuriya RC, Singhal RS. Response surface methodology for optimization of production of lovastatin by solid state fermentation. Braz J Microbiol, 2010; 41: 164-72.

[21]. Wei P, Xu Z, Cen P. Lovastatin production by Aspergillus terreus in solid-state fermentation. J Zhejiang Univ Sci A, 2007; 8: 1521-6.

[22]. Bizukojc M, Pecyna M. Lovastatin and (+)-geodin formation by Aspergillus terreus ATCC 20542 in a batch culture with the simultaneous use of lactose and glycerol as carbon sources. Eng Life Sci, 2011; 11: 272-82.

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