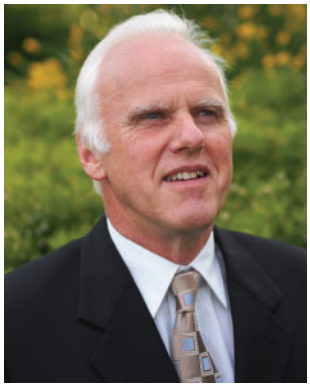
Production of statins by fungal fermentation





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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 compactin 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 synthesised 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.

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[2]. Natural statins, including lovastatin and mevastatin (commonly known as compactin) are produced by direct fungal fermentation. Semi-synthetic statins, simvastatin and pravastatin (Figure 1), are synthesised by the stereoselective hydroxylation of natural statins. Chemically synthesised statins include atorvastatin, rosuvastatin, fluvastatin and pitavastatin[3].

Discovery of statins

Compactin, discovered by Akira Endo in 1973 as a structural analogue for the HMG-CoA substrate, was produced 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 1987[4]. Pravastatin, the semi-synthetic derivative of compactin was commercialised in 1989. Simvastatin remains a commonly prescribed statin[1,5].

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[6,7]. 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[8,9]. All fungi producing lovastatin or compactin utilise the pathway shown in Figure 2[10].

Lovastatin is commercially produced by fermentation of *A. terreus* and simvastatin is produced by further chemical treatment of lovastatin usually involving direct alkylation[11,12]. Compactin is not as effective in inhibiting HMGCR as lovastatin, however, a semi-synthetic 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].

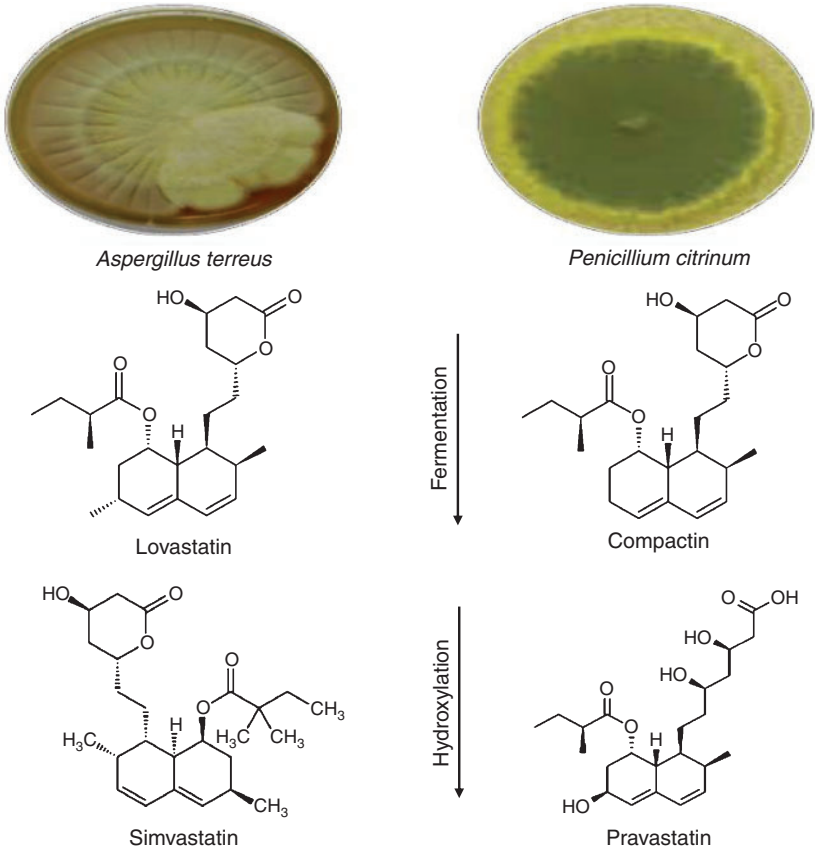

Figure 1. Production of natural and semi-synthetic statins by fungi.

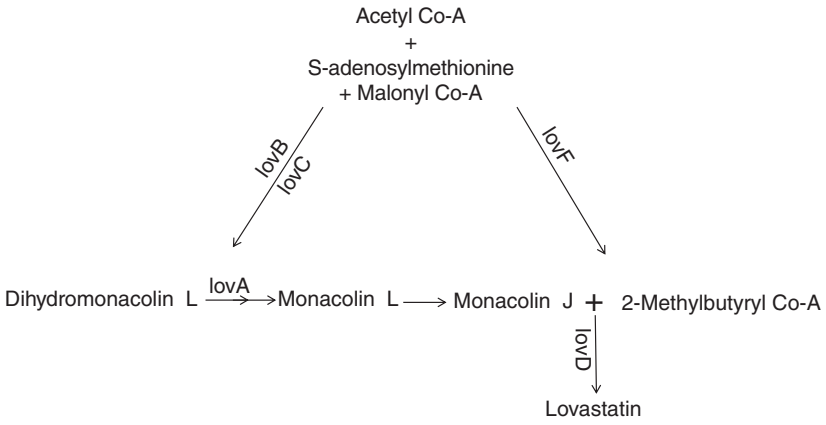

Figure 2. Pathway for the production of lovastatin in *Aspergillus terreus*.

Unlike simvastatin where conversion of lovastatin to simvastatin is a chemical reaction, the reaction for pravastatin synthesis is biotransformation[4]. Lovastatin can be directly methylated or deacylated for the synthesis of simvastatin[13]. 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[14,15]. This dual step fermentation is economically not feasible. Recent studies have reported the production of pravastatin in a single fermentative step. Enzymes responsible for the hydroxylation are genetically incorporated in the penicillin-producing fungus *Penicillium chrysogenum*. This results in efficient production of pravastatin at industrial scale[16].

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[12,17]. Some studies have also reported fed-batch fermentation that were carried

out in a bioreactor with a capacity of 1000 L. Repeated fed batch processes can also improve the productivity of desired metabolites[18].

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.

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[19]. Statins also reduce hepatic cholesterols leading to reduced gallstone formation and reduced platelet aggregation[20,21]. Recent studies have reported the role of statins in cognitive impairment after sepsis by reversing the microvascular dysfunction and reducing neuroinflammation[22]. Simvastatin has been found to reduce the incidence of neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease[23].

Acknowledgements

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Biographies

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