Strategies for the statins production in microbial cell factory:present and future

Dexun Fan[1], HuaYang Tang[1], Fengguang Zhao[1], Xiaorong Yang[1], and Shuangyan Han[1]

[1]South China University of Technology

May 22, 2023

Abstract

Statins as a lipid-lowering drug can selectively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and decrease cholesterol synthesis effectively. With the improvement of nutritional conditions, the demand for statins is increasing in global market. Due to the rapid development of modern biotechnologies, the biosynthesis of stains by microbial cell factory appears great advantages. It has the advantages of simple operation and easy separation of products. This review summarized the strategies on statins production via microbial cell factory, including both traditional fermentation culture and modern synthetic biology manufacture. Firstly, the complex fermentation parameters and process control technology on submerged fermentation (SmF) and solid-state fermentation (SSF) were introduced in detail. Especially, the possibility of recoverable agricultural wastes/(Biomass) as fermentation substrate on solid-state fermentation to produce statins was emphasized. Besides, metabolic engineering strategies to construct robust engineering strains and strains evolution were also discussed. The review highlights the potential and challenge of microbial cell factory to yield the statins. Thus, it will facilitate the production of statins in more green production mode.

Strategies for the statins production in microbial cell factory:present and future

Dexun Fan[1], Huayang Tang[1], Xiaorong Yang[1], Fengguang Zhao[2], Shuangyan Han[1]*

[1]Guangdong Key Laboratory of Fermentation and Enzyme Engineering, School of Biology and Biological Engineering, South China University of Technology, Guangzhou, China, [2]School of Light Industry and Engineering, South China University of Technology, Guangzhou, China

***Correspondence: Shuangyan Han (*syhan@scut.edu.cn*)**

Abstract

Statins as a lipid-lowering drug can selectively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and decrease cholesterol synthesis effectively. With the improvement of nutritional conditions, the demand for statins is increasing in global market. Due to the rapid development of modern biotechnologies, the biosynthesis of stains by microbial cell factory appears great advantages. It has the advantages of simple operation and easy separation of products. This review summarized the strategies on statins production via microbial cell factory, including both traditional fermentation culture and modern synthetic biology manufacture. Firstly, the complex fermentation parameters and process control technology on submerged fermentation (SmF) and solid-state fermentation (SSF) were introduced in detail. Especially, the possibility of recoverable agricultural wastes/(Biomass) as fermentation substrate on solid-state fermentation to produce statins was emphasized. Besides, metabolic engineering strategies to construct robust engineering strains

and strains evolution were also discussed. The review highlights the potential and challenge of microbial cell factory to yield the statins. Thus, it will facilitate the production of statins in more green production mode.

KEYWORDS : Statins; strategies; microorganism; fermentation; engineering strains

Introduction

Hypercholesterolemia is one of the leading causes of death from cardiovascular disease in humans. Only one-third of the total body cholesterol is diet derived, two-thirds is synthesized directly from intracellular precursors 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) (Alberts, Chen et al. 1980, Breedlove and Hedrick 1999). Statins drugs can selectively inhibit HMG-CoA reductase thus reducing lipids synthesis significantly as well as giving play to multiple biological effects such as inhibiting atherosclerosis, thrombosis and alleviating rejection reaction, treating osteoporosis, anti-tumor, etc. (Figure 1) (Cummings and Bauer 2000, A. Massy and Guijarro 2001, Barrios-González and Miranda 2010, Osmak 2012). Statins block an early step, the conversion of HMG-CoA to mevalonate, which reducing cholesterol synthesis precursors, thus directly affecting the synthesis of cholesterol (such as the reduce of LDL and the increase of HDL) (A. Massy and Guijarro 2001, Adhyaru and Jacobson 2018). And the reduction of farnesyl pyrophosphate (farnesyl-PP) and geranylgeranyl pyrophosphate (geranylgeranyl-PP) interferes with protein isoprene (the binding of lipid isoprene to proteins), thereby affecting the normal function of small glutamyl transpeptidases (GTPases) (such as Ras, Rho, Rac and Rab) in the osteoclasts, which may lead to osteoporosis, senile dementia and so on(Rikitake and Liao 2005, Binnington, Nguyen et al. 2015, Petek, Villa-Lopez et al. 2018, Healy, Berus et al. 2020). From January 1, 2002, to December 31, 2018, an average of 21.35 million statins were purchased annually, with an average total annual cost of $24.5 billion in the US (Lin, Baumann et al. 2021).

Statins can be produced through microbial synthesis and chemical synthesis. There are totally four statins which can be produced by microorganism cells, lovastatin (Alberts, Chen et al. 1980), compactin (Endo, Kuroda et al. 1976), pravastatin, simvastatin. Meanwhile, lovastatin and simvastatin are first-generation statins. Lovastatin from*Aspergillus* and compactin from *Penicillium* are two pure natural statins till now. Simvastatin can be synthesized by the precursor monacolin J, a hydrolysate of lovastatin. Pravastatin can be obtained by stereoselective hydroxylation in the fermentation of microorganism using compactin as precursor. Fluvastatin, atorvastatin, rosuvastatin and pitavastatin are fully synthetic statins (Jahnke 2007). Pravastatin and fluvastatin belong to the second-generation of statins. Atorvastatin, rosuvastatin and pitavastatin are the third-generation statins. Their chemical structures are quite different from natural statins (Table 1) and couldn't be produced by microbiology technology so far (Zhou, Curtis et al. 2019). Although there are many types of statins on the market, the first generation of statins produced by microbial cells still maintain a good trend in the world market. Although there is a strong commercial demand for statins, their production is usually at low levels in native producers from rare natural sources. The small quantities and poor purities limit the scale-up of stains production through chemical synthesis (Tartaggia, Fogal et al. 2016). Today, statins are mainly produced through microbial submerged fermentation (SmF) and solid-state fermentation (SSF) (Pawlak and Bizukojc 2013, Gonciarz, Kowalska et al. 2016). However, fermentation using native*A. terreus* usually poses some problems such as a long culture period, difficult manipulation, and multiple byproducts. Synthetic biology has many advantages compared to chemical synthesis, such as carbon neutral, sustainable, low cost, etc. With the development of synthetic biology, reconstruction of biosynthetic pathways in chassis organisms has been proved to be a possible solution to these problems(Ro, Paradise et al. 2006, Galanie, Thodey et al. 2015). Therefore, a growing number of researchers are looking into using microbial cell factories to yield statins (Ro, Paradise et al. 2006).

Here, we reviewed the strategies of microbial cell fermentation to produce statins in recent 20 years, mainly include submerged fermentation (SmF) and solid-state fermentation (SSF). As a vital strategy to improve statins synthesis at the cellular level, we have also reviewed findings that provide guidance on strains construction by metabolic engineering strategies and evolution. We also illustrated the great potential and challenges of producing statins through microbial cell factories.

Microbial cell fermentation to produce statins

Now, statins are produced mainly through microbial cell fermentation, mainly divided into submerged fermentation (SmF) and solid-state fermentation (SSF). According to the reports, three most aspects including medium, dissolved oxygen and other effects, respectively, affecting the submerged fermentation (SmF). The medium and other effects are two most aspects that affecting solid-state fermentation (SSF) (Figure 2).

2.1 Statins produced in SmF

Submerged fermentation (SmF) technology has the advantages of short cycle, low cost and high yield, and the purification of products is easier. Medium components especially carbon, nitrogen sources and inorganic salt are most influential aspects for statins production. The dissolved oxygen in the medium also has a great influence on the synthesis of statins. There are also many other effects such as Antibiotics, surfactants, the age of selected spores and fed-batch fermentation, affecting the production of statins. Submerged fermentation for statins biosynthesis was summarized in Table 2.

2.1.1 Medium

Carbon sources and nitrogen sources

Carbon and nitrogen sources are essential for microbial growth. It has a great influence on the synthesis of lovastatin. *A. terreus* is the native strain that produces lovastatin. Therefore, most people focus on optimizing carbon and nitrogen sources using *A. terreus* ATCC 20542. Ansari et al.(Ansari, Jalili et al. 2018) showed that 64 g/L syrup carbohydrates, 15 g/L yeast extract can lead to the lovastatin titer of 105.6 mg/L using *A. terreus* ATCC 20542. Batch cultures were performed in a 2.5-L working volume bioreactor and led to the lovastatin titer of 241.1 mg/L during 12 days *A. terreus* ATCC 20542. Rollini et al.(Rollini and Manzoni 2006) showed soybean peptone generally allowed the best lovastatin yields to be achieved (250–280 mg/L) by *A. terreus*ATCC 20542, particularly in the presence of soybean and peanut flours. Vegetable oil as sole carbon source and supplemental carbon source has effects on the fermentation of lovastatin by *A. terreus* . Sripalakit et al.(Sripalakit and Saraphanchotiwitthaya 2020) showed that all selected vegetable oils increased yields by at least two-fold. Especially, when 1% w/v coconut oil was added, the highest yield was 87.18 g/L using *A. terreus* ATCC 20542, approximately 11-fold compared to the oil-free control group. Hajjaj et al.(Hajjaj, Niederberger et al. 2001) found that a threefold-higher specific productivity was found with the defined medium on glucose and glutamate, compared to growth on complex medium with glucose, peptonized milk, and yeast extract using *A. terreus* Thom ATCC 74135. Oliveira et al.(Oliveira, Paulo et al. 2021) showed that 60 g/L soluble starch, 15 g/L soybean flour led to producing 100.86 mg/L lovastatin using *A. terreus* URM 5579. Kaur et al.(Kaur, Kaur et al. 2010) optimized the culture-medium parameters of *A. terreus* GD_{13}. They found it can lead to the maximal lovastatin titer of 1342 mg/L when the initial C:N ratio in the culture medium was 37:1, which was 7-fold compared to the titer obtained under unoptimized conditions. Submerged fermentation of agricultural waste for fermentation substrate to produce statins can not only protect the environment, but also recycle resources. Srinivasan et al. (Srinivasan, Thangavelu et al. 2022) used*A. terreus* KPR 12 to ferment the sago processing wastewater, getting 429.98 mg/L lovastatin. Medium optimization for other strains has also been reported. Atli et al. (Atli, Yamaç et al. 2013) found 30 g/L glucose, 10 g/L yeast extract can lead to 114.82 mg/L lovastatin using *P. ostreatus* OBCC 1031.

Carbon sources, nitrogen sources also have a great influence on the synthesis of compactin. Chakravarti et al.(Chakravarti and Sahai 2002) optimized the medium for compactin production by *P. citrinum* NCIM 768, lead to the maximum titer increased to 490 mg/L. Ahmad et al.(Ahmad, Panda et al. 2010) showed that glycerol, peptone, yeast extract improved the titer of compactin to 589.3 mg/L using*P.citrinum* MTCC 1256. Syed et al.(Syed and Rajasimman 2015) optimized medium on the production of compactin by *A. terreus* , lead to the titer of compactin increased to 701 mg/L. Jekkel et al.(Kónya, Jekkel et al. 1998) showed that 7.0% glucose, 1.0% yeast extract led to the titer of compactin to 390-410 mg/mL using *P. citrinum* MTCC 1256.

Inorganic salts

Inorganic salts are not only nutrients for microbial growth, but also can participate in the building blocks of microbial cells and enzymes. Therefore, inorganic salts have a certain effect on the biosynthesis of lovastatin. Rahim et al.(Abd Rahim, Lim et al. 2019) showed that the yield of lovastatin increased by 282% to 25.52 mg/L when the medium have KH_2PO_4, $MgSO_4{\cdot}7H_2O$, NaCl and $ZnSO_4{\cdot}7H_2O$ using *A. terreus* ATCC 20542. Jia et al.(Jia, Zhang et al. 2009) showed that Fe^{2+}, Ca^{2+}, Zn^{2+}, Mg^{2+} and Mn^{2+} can promote lovastatin synthesis and cell growth. In the presence of 2mM and 5mM Zn^{2+}, the highest titer was 49.2±1.4 mg $gDCW^{-1}$, a 14.4-fold increased using *A. terreus*ATCC 20542. Sayyad et al.(Sayyad, Panda et al. 2007) optimized *M. purpureus* MTCC 369 to produce lovastatin. In the medium containing 3.86 g/L NH_4Cl, 1.73 g/L KH_2PO_4, 0.86 g/L $MgSO_4{\cdot}7H_2O$ and 0.19 g/L $MnSO_4{\cdot}H_2O$, the maximum titer of lovastatin increased to 351 mg/L.

2.1.2 Dissolved Oxygen (DO)

The dissolved oxygen in Submerged fermentation medium has great influence on the synthesis of statins. Gonciarz et al.(Gonciarz and Bizukojc 2014) added 10μm of talc particles in the medium to decreases fungal pellet size to increase the oxygen saturation of the broth, which lead to lovastatin titer increased by 3.5-fold to exceed then 120 mg/L. The optimal continuous feed batch increased to 250 mg/L(Gonciarz, Kowalska et al. 2016). Lai group(Lai, Tsai et al. 2005) found that the 5-L fermenter increased lovastatin titer by 38% when the dissolved oxygen (DO) was controlled at 20%. When the medium temperature was reduced from 28 to 23 , the titer of lovastatin was further increased by 25%, reaching 572 mg/L. Ansari et. al(Ansari, Jalili et al. 2019) showed that the highest titer of lovastatin (443 mg/L) were obtained at air bubbles diameter of 0.18 cm. The main reason is that the diameter of the bubble directly affects the concentration of dissolved oxygen.

In fact, reactive oxygen species (ROS) produced during fermentation have an effect on lovastatin biosynthesis The addition of N-Acetyl-L-cysteine (NAC), which reduces reactive oxygen species, can reduce lovastatin production. On the contrary, the addition of H_2O_2, which promotes reactive oxygen species (ROS) production, leads to lovastatin biosynthesis(Miranda, Gómez-Quiroz et al. 2014).

Redox potential of fermenters can also affect lovastatin synthesis(Pawlak and Bizukojc 2013). In the process with the highest redox potential levels maximum lovastatin concentration was equal to 83.8 mg/L, while at the lowest redox level it did not reach 67 mg/L.

2.1.3 Other effects

Antibiotics

Jia et al.(Jia, Zhang et al. 2010) added different polyketide antibiotics to the medium by *A. terreus* ATCC 20542, which leaded to improve lovastatin titer by 20-25%, such as tylosin, erythromycin, tetracycline, daunorubicin. Especially, lovastatin titer reached 952.7±24.3 mg/L at the initial stage of lovastatin synthesis with the addition of 50 mg/L tylosin, increased by 42% and 22.2%, respectively.

Surfactants

Chakravarti et al.(Chakravarti and Sahai 2002) cultured the mutant strain of *P. citrinum* NCIM 768 in chemically-defined medium, producing 145 mg/L compactin. The titer of compactin was increased to 175 mg/L by adding surfactant tween 80 into the medium. Choi group(Choi, Cho et al. 2004) showed that compactin titer was 1200 mg/L after 10 days of fermentation with 1.5 g/L triton X-100, increased by 2-fold.

Age of selected spores

The age of selected spores not only has a great influence on the growth state, but also affects the expression level of cells. Porcel et al.(Porcel, López et al. 2006) found a higher titer for lovastatin production by using older spores. The titer of lovastatin increased by 52% to 186.5±20.1 mg/L when the age of inoculated spores increased from 9 days to 16 days.

Fed-batch

Pecyna et al.(Pecyna and Bizukojc 2011) showed that the application of glycerol feed, when lactose is the initial substrate, leads to the appreciable lovastatin concentration in the broth (122.4 mg/L), nevertheless the abundant (+)-geodin level is at the same time obtained (255.5 mg/L). The cultures with glycerol as the initial substrate and fed with lactose produce less lovastatin and (+)-geodin. The application 3of the various combined glycerol and/or lactose feeds allows for improving lovastatin production up to 161.8 mg/L. Porcel et al.(Porcel, López et al. 2008) showed that semi-continuous operation enhanced productivity of lovastatin by 315% compared with a conventional batch operation.

2.2 Statins produced in SSF

Solid-state fermentation (SSF) has the advantages of simple operation, low energy consumption, easy control of fermentation process, relatively low requirement for sterility, and not easy to occur large area pollution. The main media of solid-state fermentation (SSF) are agricultural raw materials, including corn, rice, sorghum, barley and so on. Biomass is a kind of renewable and clean energy. The rational, efficient development and utilization of biomass is also a hot spot for Solid-state fermentation (SSF) to produce statins. In fact, the carrier used in the fermentation process and the surface wind speed can also affect the fermentation of microorganisms to produce statins. Solid-state fermentation for statins biosynthesis was summarized in Table 3.

2.2.1 Medium

Different medium has great influence on lovastatin production by microbial solid-state fermentation. Valera et al.(Valera, Gomes et al. 2005) found that wheat bran was to be the most suitable substrate to yield 16.65 mg/g lovastatin in aerated stirred beds after 6 days of fermentation by*A. flavipes* BICC 5174. Atlı et al. (Atlı, Yamaç et al. 2015) showed that a maximum lovastatin titer of 139.47 mg/g was achieved by the fermentation of 5 g of barley, 1–2 mm particle diam, at 28 °C. Subhagar et al.(Subhagar, Aravindan et al. 2009) showed barley, long grain rice and sago starch were the suitable substrates producing. The maximum titer of lovastatin are 193.7 mg/g, 190.2 mg/g and 180.9 mg/g,respectively. Suraiya et al.(Suraiya, Kim et al. 2018) showed that glucose had the greatest influence on the production of lovastatin. Under the optimal fermentation parameters, the average titer of lovastatin reached 13.98 mg/gds using *M. purpureus* KCCM 60168. Pansuriya et al.(Pansuriya and Singhal 2010) also did this work. The titer of lovastatin was to 3.723 mg/g by *A. terreus* UV 1718 using solid-state fermentation when optimizing the fermentation parameters.

Different medium also has great influence on compactin production by microbial solid-state fermentation. Shaligram et al.(Shaligram, Singh et al. 2008) showed that the optimal production of compactin was 0.771 mg/gds with the addition of various supplements (glycerin, etc.) by*P. brevicompactum* WA 2315. The titer of compactin was increased to 0.815 mg/gds when the pH of the supplement solution was 7.5. Shaligram et al.(Shaligram, Singh et al. 2008) optimized the synthesis of compactin by *P. brevicompactum* WA 2315. The final titer of compactin was increased from 0.45 mg/gds to 1.25 mg/gds by adding glycerol during fermentation. Syed et al.(Syed, Rajendran et al. 2014) showed that the combinations of the substrates with 1.5 g of green peas, 1.5 g of millet and 1.5 g of ragi gave maximum production of 389.34 mg/gds compactin by *A. terreus* MTCC 279.

Biomass is a kind of renewable and clean energy. The rational, efficient development and utilization of agricultural waste as the substrate of solid-state fermentation (SSF) to produce statins can not only save production cost effectively, but also realize the effective utilization of resources. Iewkittayakorn et al.(Iewkittayakorn, Kuechoo et al. 2020) showed that the titer of lovastatin reached the highest at 0.99 mg/g after 14 days of fermentation with soybean sludge as substrate by adding addition palm oil. Javed et al.(Javed, Bukhari et al. 2016) studied the production of compactin by solid-state fermentation of with bagasse as substrate by *A. terreus* GCBL-03. Bagasse was pretreated by potassium hydroxide readily become available to microorganism, leading to 30.63+-1.24 mg/100mL.

2.2.2 Other effects

Artificial inert support

Banos et al.(Banos, Tomasini et al. 2009) used high-density polyurethane foam (PUF) as an inert support to produce lovastatin by SSF. Results showed that the titer of lovastatin in PUF solid-state fermentation is two-fold higher than that of the known solid-state fermentation system of bagasse. And the titer of lovastatin on PUF is more than 15-fold higher than that of submerged fermentation.

Superficial air velocity

Kumar et al.(Kumar, Srivastava et al. 2014) studied the effect of superficial air velocity on lovastatin production from *A. terreus*PL 10 using wheat bran and wheat straw in a 1200-L packed bed reactor. Results showed a maximum lovastatin production of 1.86 mg/g when the reactor was operated using 0.19 vvm airflow rate (23.37 cm/min superficial air velocity).

Co-cuture

In fact, co-culture technique has also been introduced to improve the yield of lovastatin. Panda et al.(Panda, Javed et al. 2010) co-cultured*M. purpureus* MTCC 369 and *M. ruber* MTCC 1880, which lead to maximum lovastatin production of 2.83 mg/g.

Improving statins production by engineering strains

Traditional fermentation culture production of statins usually poses some problems such as a long culture period, difficult manipulation, and multiple byproducts. With the rapid development of synthetic biology, the construction of engineering strains for the production of statins may be a major strategy for present and future statin production. At the same time, the improvement of metabolic engineering strategies should be rational pathway design and modification. All strains are modified to meet production requirements. We summarized the metabolic engineering strategies from the perspectives of heterologous expression of genes, modification of regulatory proteins, inhibiting by-product synthesis, respectively. In contrast to metabolic engineering strategies, evolution of strains is another alternative to improve the production. Engineering strains strategies for statins biosynthesis was summarized in Table 4.

3.1 Heterologous expression of genes

Heterologous expression of genes is a common strategy in synthetic biology. Heterologous expression of genes strategies to improve statins production are described in the box at the upper left (Figure 3 A-C).*S. cerevisiae* is very suitable for heterologous expression of genes. Bond et al.(Bond and Tang 2019) introduced six heterosynthetic genes into *S. cerevisiae*BY4741 and adding the acyl-donor dimethylbutyryl-S-methyl mercaptopropionate (DMB-SMMP) into the culture medium. Regulating the copy number of *lovA* and introducing the gene *npgA* and in situ chemical lysis of cell wall, lead to 55 mg/L simvastatin. Liu et al.(Liu, Tu et al. 2018) introduced lovastatin synthesis gene into*P. pastoris* GS115. Using dihydromonacolin L as a metabolic node, the synthetic pathway is divided into upstream and downstream modules. Finally, the optimal co-culture strategy was selected by bioreactor fermentation, lead to 250.8 mg/L lovastatin (Figure 3(A)).

Currently, industrial production of simvastatin acid (SVA) is a multistep process starting from the natural product lovastatin. Monacolin J can be obtained by alkaline hydrolysis of lovastatin. Chemical method for transformation of monacolin J to simvastatin was generally divided into three steps, including hydroxyl group protection, reesterification, and deprotection. The processes from lovastatin to simvastatin are complicated, laborious, and environmentally unfriendly(Askin, Verhoeven et al. 1991, Xie, Watanabe et al. 2006, Huang, Liang et al. 2017). Monacolin J biosynthetic gene cluster were integrated into the genome of *A. niger* CBS513.88(Zeng, Zheng et al. 2022) which processes strong promoters and suitable integration sites, lead to 92.90 mg/L monacolin J. Optimizing culture conditions and adding precursors, improved the titer to 142.61 mg/L. Liang et al.(Liang, Huang et al. 2018) achieved single-step in vivo production of monacolin J by using lovastatin hydrolase (PcEST) in *A. terreus* HZ01 (Figure 3(B)). After modification of PcEST, cell activity

was increased by 18-dold, which greatly promoted hydrolysis of lovastatin to monacolin J, which also laid a foundation for industrial production of simvastatin.

Compactin synthetic gene cluster has not been fully characterized. The function of specific genes of compactin synthetic is unclear. However, there are still some reports of compactin production in engineered strains. Abe et al.(Abe, Suzuki et al. 2002) improved the synthesis of compactin by adding some gene clusters related to compactin synthesis in *P. piltrinum* No.41520. Baba et al.(Baba, Abe et al. 2009) improved the titer of compactin by adding complete gene clusters in *P. piltrinum* No.41520, lead to the titer of compactin increase by 50%. These results indicate that increasing gene copy numbers can promote high titer of compactin.

Pravastatin is mostly produced by microbial fermentation using compactin or compactin sodium as substrate. Screening strains with high conversion rate is the key to obtain high yield of pravastatin. Lin et al.(Lin, Tang et al. 2011) isolated a strain and further identified as *P. carboxydivorans* PAH4. In the medium of 1 mg/ml compactin sodium, the conversion rate of pravastatin reached 68%. The results of this study suggested *P. carboxydivorans* PAH4 could be considered a candidate for the production of pravastatin on an industrial scale. Ahmad et al.(Ahmad, Mujeeb et al. 2013) tested the bioconversion of compactin to pravastatin by three *A.* species, named *A. livida* MTCC 1382, *A. macra* MTCC 2559, and *A. madurae* MTCC 1220. Bioconversion by *A. macra* MTCC 2559 was highest (87 %) in the yeast extract-amended medium. Park et al.(Park, Lee et al. 2003) isolated *Streptomyces sp.* Y-110 from soil. In batch culture, the maximum titer was 340 mg/L. By adding compactin to the medium intermittently, the titer was increased to 1000 mg/L. McLean et al.(McLean, Hans et al. 2015) introduced the compactin pathway into the beta-lactam-negative *P. chrysogenum* DS50662, a new cytochrome P450 (P450 or CYP) was isolated to catalyze the final compactin hydroxylation. They evolved the CYP enzyme to reverse stereoselectivity, lead to more than 6 g/L pravastatin at a pilot production scale (Figure 3(C)).

3.2 Modification of regulatory proteins

Modifying the regulatory element proteins strategies to improve statins production are described in the box at the upper right (Figure 3 D, E). Liu et al.(Liu, Bai et al. 2018) overexpressed the statins pump protein TapA (a membrane protein that enables lovastatin to flow out of cells) in *P. pastoris* GS115, resulted in 419.0+-9.5 mg/L lovastatin, 46% higher than overexpression of lovastatin gene and 520% higher than single-copy strain, respectively (Figure 3(D)). They similarly modulated Trap proteins in *P. pastoris* GS115, successfully increasing monacolin J production(Bai, Liu et al. 2020). Itoh et al.(Itoh, Miura et al. 2018) knocked out the sterol regulatory element binding protein (SREBP) system, increased the lovastatin production by *A. terreus*ATCC 20542. Thus, knockout of the SREBP system should be considered significant for increasing the productivities of polyketides, such as HMG-CoA reductase inhibitors, by filamentous fungi. Lu et al.(Huang, Tang et al. 2019) overexpressed the lovastatin transcriptional regulator*love* , increased the synthetic yield of monacolin J by 52.5% (Figure 3(E)).

3.3 Inhibiting by-product synthesis

The by-product (+)-Geodin is produced when lovastatin is synthesized from *A. terreus* ATCC 20542 in glycerol culture. Hasan et al.(Hasan, Abd Rahim et al. 2019) inserted the antibiotic marker hygromycin B (*hyg*) within the *gedC* gene that encodes emodin anthrone polyketide synthase (PKS), got an *A. terreus*mutant strain ($\gamma\epsilon\delta\tilde{}\Delta$). Compared with the wild-type strain, the yield of lovastatin was increased by 80% to 113 mg/L. This study also provided a practical method for controlling carbon flux (Figure 3(F)).

3.4 Evolution of strains

Evolution of strains is a method to obtain high-yield strains. Chemical inducers and ultraviolet radiation are common methods of random mutation. Kaur et al.(Kaur, Kaur et al. 2009) induced *A. terreus*GD_{13} for three cycles to get high-yielding lovastatin*A. terreus* EM19, increased 7.5-fold to 1424 mg/L. Azeem et al.(Azeem, Arshad et al. 2020) induced *A. terreus* with ethidium bromide for a long time, which greatly improved the yield of lovastatin in solid-state fermentation. El-Bondkly et al.(El-Bondkly, El-Gendy et al. 2021) obtained 4.51 mg/gds lovastatin by solid-state fermentation of straw by *Fusarium sp.* Alaa-20. Enhancing

mutagenesis induction and three successive gene recombination of Fusarium alternium, increased the titer to 52.1 mg/gds. Dzhavakhiya et al.(Dzhavakhiya, Voinova et al. 2015) obtained a strain *S. xanthochromogenes* S33-1 that is high tolerance of compactin by multi-step random UV mutagenesis of *S. xanthochromogenes* RIA 1098. After the fermentation medium improvement, the maximum bioconversion rate of this strain has reached 91% at the daily compactin dose equal to 1 g/L and still remained high (83%) even at the doubled dose (2 g/L) (Figure 3(G)).

4、Challenges and future prospects

From the perspective of statins production strategies, most of the research is based on solid-state fermentation and submerged fermentation. Most of them are optimized for the composition of carbon source, nitrogen source and inorganic salt in the medium. Some papers have also studied the fermentation parameters and the substances produced in the fermentation process that may affect the yield of statins. It can be concluded that simply optimizing the culture medium components and fermentation parameters will not lead to significant progress in statins production. At the same time, the lack of microbial growth and catalytic activity in industrial fermenters will lead to low product yield, weak cell growth and other problems. Global screening or random mutagenesis of existing strains to obtain more adaptable strains may solve this problem(Maltsev, Maltseva et al. 2020, Chekanov, Litvinov et al. 2021). Metabolic engineering strategies have also been used to increase statins production, but these have been relatively infrequently reported. This is partly because some of the statins synthesis gene clusters have not been fully characterized(Abe, Suzuki et al. 2002, Baba, Abe et al. 2009), limiting gene modification. Therefore, a complete analysis and characterization of the statins synthesis gene cluster will further promote statins synthesis.

S. cerevisiae is an ideal host for heterologous gene expression(Novo, Bigey et al. 2009, Vatanparast and Kim 2019, Davies, Tsyplenkov et al. 2021). The mature technologies of gene editing and expression, high cell-density culture and fermentation process control made S. cerevisiae to be a very promising microorganism for statins production. The successful synthesis of simvastatin(Bond and Tang 2019) has demonstrated that*Saccharomyces cerevisiae* may be a promising microorganism for the production of statins. In particular, new statins can be synthesized by introducing exogenous synthetic genes into *S. cerevisiae* (Giugliano, Maiorino et al. 2019, Chioua and Marco-Contelles 2021). However, some challenges still exist to translate bio-statins into practical industrial applications.

In the future, major advances in statins production will depend on metabolic engineering strategies, which also need biotechnology innovation. Methods such as protein engineering, synthetic biology, metabolic engineering and fermentation engineering will be used to overcome challenges and solve biotechnology problems(Liu, Xue et al. 2022). Synthetic biology and systems biology tools help to explore and construct shorter alternatives to the classical statins synthesis pathway(2012, Nielsen and Pronk 2012). Protein engineering and structural biology tools are needed to improve transformation efficiency and mitigate the inhibition of key intermediates and end products. Adaptively directed evolution of enzymes has also benefited from advances in protein engineering. Therefore, through the further study of metabolic engineering strategies, the production of statins will make significant progress. Compared to review papers on statins production previously published(Manzoni and Rollini 2002, Barrios-González and Miranda 2010), We describe the strategies of statins synthesis in more detail. And we outlook the challenges and possible solutions of statins synthesis in more detail and comprehensively. Overall, statins biosynthesis is a worthy-studied theme, as statins still have high application and value.

Author Information

Corresponding Author

*E-mail: syhan@scut.edu.cn

ORCID

Shuangyan Han: 0000-0002-9869-5829

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Author Contributions

All authors contributed to the background research and writing of the article, as well as the editing. In addition, all authors have read and approved the final version of this manuscript.

Conflicts Of Interest

The authors declare that there are no conflicts of interest.

Funding Information

This work was supported by the National Key R&D Program of China (2021YFC2102700).

Reference

(2012). "Metabolic Engineering and Synthetic Biology in Strain Development." *ACS Synthetic Biology* **1** (11): 491-492.

A. Massy, Z. and C. Guijarro (2001). "Statins: effects beyond cholesterol lowering." *Nephrology Dialysis Transplantation***16** (9): 1738-1741.

Abd Rahim, M. H., E. J. Lim, H. Hasan and A. Abbas (2019). "The investigation of media components for optimal metabolite production of Aspergillus terreus ATCC 20542." *Journal of Microbiological Methods* **164** : 105672.

Abe, Y., T. Suzuki, T. Mizuno, C. Ono, K. Iwamoto, M. Hosobuchi and H. Yoshikawa (2002). "Effect of increased dosage of the ML-236B (compactin) biosynthetic gene cluster on ML-236B production in Penicillium citrinum." *Molecular Genetics and Genomics* **268** (1): 130-137.

Abe, Y., T. Suzuki, C. Ono, K. Iwamoto, M. Hosobuchi and H. Yoshikawa (2002). "Molecular cloning and characterization of an ML-236B (compactin) biosynthetic gene cluster in Penicillium citrinum." *Molecular Genetics and Genomics* **267** (5): 636-646.

Adhyaru, B. B. and T. A. Jacobson (2018). "Safety and efficacy of statin therapy." *Nature Reviews Cardiology* **15** (12): 757-769.

Ahmad, A., M. Mujeeb, R. Kapoor and B. P. Panda (2013). "In situ bioconversion of compactin to pravastatin by Actinomadura species in fermentation broth of Penicillium citrinum." *Chemical Papers***67** (6): 667-671.

Ahmad, A., B. Panda and M. M. (2010). "Screening of nutrient parameters for mevastatin production by Penicillium citrinum MTCC 1256 under submerged fermentation using the Plackett-Burman design." **2** (1): 44-46.

Alberts, A. W., J. Chen, G. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Albers-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch and J. Springer (1980). "Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent." **77** (7): 3957-3961.

Ansari, F. J., H. Jalili, M. Bizukojc and A. Amrane (2018). "Optimization of date syrup as a novel medium for lovastatin production by Aspergillus terreus ATCC 20542 and analyzing assimilation kinetic of carbohydrates." *Annals of Microbiology* **68** (6): 351-363.

Ansari, S., H. Jalili, M. Bizukojc and A. Amrane (2019). "Influence of the construction of porous spargers on lovastatin production by Aspergillus terreus ATCC 20,542 in a laboratory bubble column." *Bioprocess and Biosystems Engineering* **42** (7): 1205-1213.

Askin, D., T. R. Verhoeven, T. M. H. Liu and I. Shinkai (1991). "Synthesis of synvinolin: extremely high conversion alkylation of an ester enolate." *The Journal of Organic Chemistry* **56** (16): 4929-4932.

Atli, B., M. Yamaç and Z. Yildiz (2013). "Optimization of Submerged Fermentation Conditions for Lovastatin Production by the Culinary-Medicinal Oyster Mushroom, Pleurotus ostreatus (Higher Basidiomycetes)."**15** (5): 487-495.

Atlı, B., M. Yamaç, Z. Yıldız and O. S. Isikhuemnen (2015). "Enhanced production of lovastatin by Omphalotus olearius (DC.) Singer in solid state fermentation." *Revista Iberoamericana de Micología***32** (4): 247-251.

Azeem, M., M. Arshad, S. Mahmood, S. Abrar, A. F. Zahoor, S. Javed, B. Tariq and K. Hayyat (2020). "Development of One Pot Strategy for Hyper Production and In Vivo Evaluation of Lovastatin." **25** (19): 4380.

Baba, S., Y. Abe, T. Suzuki, C. Ono, K. Iwamoto, T. Nihira and M. Hosobuchi (2009). "Improvement of compactin (ML-236B) production by genetic engineering in compactin high-producing Penicillium citrinum." *Applied Microbiology and Biotechnology* **83** (4): 697-704.

Bai, C., Y. Liu, X. Chen, Z. Qian, H. Liu, X. Zhou, Y. Zhang and M. Cai (2020). "Fungal statin pump protein improves monacolin J efflux and regulates its production in Komagataella phaffii." *Bioresources and Bioprocessing* **7** (1): 32.

Baños, J. G., A. Tomasini, G. Szakács and J. Barrios-González (2009). "High lovastatin production by Aspergillus terreus in solid-state fermentation on polyurethane foam: An artificial inert support." *Journal of Bioscience and Bioengineering* **108** (2): 105-110.

Barrios-González, J. and R. U. Miranda (2010). "Biotechnological production and applications of statins." *Applied Microbiology and Biotechnology* **85** (4): 869-883.

Binnington, B., L. Nguyen, M. Kamani, D. Hossain, D. L. Marks, M. Budani and C. A. Lingwood (2015). "Inhibition of Rab prenylation by statins induces cellular glycosphingolipid remodeling." *Glycobiology***26** (2): 166-180.

Bond, C. M. and Y. Tang (2019). "Engineering Saccharomyces cerevisiae for production of simvastatin." *Metabolic Engineering***51** : 1-8.

Breedlove, C. and H. Hedrick (1999). "Reenvisioning Medical Education for the New MillenniumCall for Papers." *JAMA* **282** (22): 2171-2171.

Chakravarti, R. and V. Sahai (2002). "A chemically-defined medium for production of compactin by Penicillium citrinum." *Biotechnology Letters* **24** (7): 527-530.

Chakravarti, R. and V. Sahai (2002). "Optimization of compactin production in chemically defined production medium by Penicillium citrinum using statistical methods." *Process Biochemistry***38** (4): 481-486.

Chekanov, K., D. Litvinov, T. Fedorenko, O. Chivkunova and E. Lobakova (2021). "Combined Production of Astaxanthin and β-Carotene in a New Strain of the Microalga Bracteacoccus aggregatus BM5/15 (IPPAS C-2045) Cultivated in Photobioreactor." **10** (7): 643.

Chioua, M. and J. Marco-Contelles (2021). "Synthesis of New Statin Derivatives." **6** (47): 13633-13635.

Choi, D., K. Cho, W. S. Cha and S. R. Ryu (2004). "Effect of triton X-100 on compactin production fromPenicillium citrinum." *Biotechnology and Bioprocess Engineering* **9** (3): 171-178.

Cummings, S. R. and D. C. Bauer (2000). "Do Statins Prevent Both Cardiovascular Disease and Fracture?" *JAMA* **283** (24): 3255-3257.

Davies, M. E., D. Tsyplenkov and V. J. J. Martin (2021). "Engineering Yeast for De Novo Synthesis of the Insect Repellent Nepetalactone." *ACS Synthetic Biology* **10** (11): 2896-2903.

Dzhavakhiya, V. V., T. M. Voinova, E. V. Glagoleva, D. V. Petukhov, A. I. Ovchinnikov, M. I. Kartashov, B. B. Kuznetsov and K. G. Skryabin (2015). "Strain Improvement of Streptomyces xanthochromogenes RIA 1098 for Enhanced Pravastatin Production at High Compactin Concentrations." *Indian Journal of Microbiology* **55** (4): 440-446.

El-Bondkly, A. A. M., M. M. A. A. El-Gendy and A. M. A. El-Bondkly (2021). "Construction of Efficient Recombinant Strain Through Genome Shuffling in Marine Endophytic Fusarium sp. ALAA-20 for Improvement Lovastatin Production Using Agro-Industrial Wastes." *Arabian Journal for Science and Engineering* **46** (1): 175-190.

Endo, A., M. Kuroda and K. Tanzawa (1976). "Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme a reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity." **72** (2): 323-326.

Galanie, S., K. Thodey, I. J. Trenchard, M. Filsinger Interrante and C. D. Smolke (2015). "Complete biosynthesis of opioids in yeast." *Science* **349** (6252): 1095-1100.

Giugliano, D., M. I. Maiorino, M. Longo and K. Esposito (2019). "Are gliflozins the new statins for diabetes?" *Diabetes Research and Clinical Practice* **153** : 191-193.

Gonciarz, J. and M. Bizukojc (2014). "Adding talc microparticles to Aspergillus terreus ATCC 20542 preculture decreases fungal pellet size and improves lovastatin production." **14** (2): 190-200.

Gonciarz, J., A. Kowalska and M. Bizukojc (2016). "Application of microparticle-enhanced cultivation to increase the access of oxygen to Aspergillus terreus ATCC 20542 mycelium and intensify lovastatin biosynthesis in batch and continuous fed-batch stirred tank bioreactors." *Biochemical Engineering Journal* **109** : 178-188.

Hajjaj, H., P. Niederberger and P. Duboc (2001). "Lovastatin Biosynthesis by Aspergillus terreus in a Chemically Defined Medium."**67** (6): 2596-2602.

Hasan, H., M. H. Abd Rahim, L. Campbell, D. Carter, A. Abbas and A. Montoya (2019). "Improved lovastatin production by inhibiting (+)-geodin biosynthesis in Aspergillus terreus." *N Biotechnol* **52** : 19-24.

Healy, A., J. M. Berus, J. L. Christensen, C. Lee, C. Mantsounga, W. Dong, J. P. Watts, M. Assali, N. Ceneri, R. Nilson, J. Neverson, W.-C. Wu, G. Choudhary and A. R. Morrison (2020). "Statins Disrupt Macrophage Rac1 Regulation Leading to Increased Atherosclerotic Plaque Calcification." *Arteriosclerosis, Thrombosis, and Vascular Biology* **40** (3): 714-732.

Huang, X., Y. Liang, Y. Yang and X. Lu (2017). "Single-step production of the simvastatin precursor monacolin J by engineering of an industrial strain of Aspergillus terreus." *Metabolic Engineering***42** : 109-114.

Huang, X., S. Tang, L. Zheng, Y. Teng, Y. Yang, J. Zhu and X. Lu (2019). "Construction of an Efficient and Robust Aspergillus terreus Cell Factory for Monacolin J Production." *ACS Synthetic Biology***8** (4): 818-825.

Iewkittayakorn, J., K. Kuechoo, Y. Sukpondma, V. Rukachaisirikul, S. Phongpaichit and W. Chotigeat (2020). "Lovastatin Production by Aspergillus sclerotiorum Using Agricultural Waste." *Food Technol Biotechnol* **58** (2): 230-236.

Itoh, H., A. Miura, M. Matsui, T. Arazoe, K. Nishida, T. Kumagai, M. Arita, K. Tamano, M. Machida and T. Shibata (2018). "Knockout of the SREBP system increases production of the polyketide FR901512 in

filamentous fungal sp. No. 14919 and lovastatin in Aspergillus terreus ATCC20542." *Applied Microbiology and Biotechnology***102** (3): 1393-1405.

Jahnke, W. (2007). "Perspectives of biomolecular NMR in drug discovery: the blessing and curse of versatility." *Journal of Biomolecular NMR* **39** (2): 87-90.

Javed, S., A. S. Bukhari, M. Ali and R. Sajjad ur (2016). "Estimation of Antifungal Activity of Mevastatin Produced by Aspergillus terreus GCBL-03 on pretreated substrate in solid state fermentation." *Current Pharmaceutical Biotechnology* **17** (3): 291-298.

Jia, Z., X. Zhang, Y. Zhao and X. Cao (2009). "Effects of divalent metal cations on lovastatin biosynthesis from Aspergillus terreus in chemically defined medium." *World Journal of Microbiology and Biotechnology* **25** (7): 1235-1241.

Jia, Z., X. Zhang, Y. Zhao and X. Cao (2010). "Enhancement of Lovastatin Production by Supplementing Polyketide Antibiotics to the Submerged Culture of Aspergillus terreus." *Applied Biochemistry and Biotechnology* **160** (7): 2014-2025.

Kaur, H., A. Kaur, H. Saini and B. Chadha (2009). "Screening and selection of lovastatin hyper-producing mutants of Aspergillus terreus using cyclic mutagenesis %J Acta Microbiologica et Immunologica Hungarica." **56** (2): 169-180.

Kaur, H., A. Kaur, H. Saini and B. Chadha (2010). "Response surface methodology for lovastatin production by Aspergillus terreus GD13 strain %J Acta Microbiologica et Immunologica Hungarica." **57** (4): 377-391.

Kónya, A., A. Jekkel, J. Sütő and J. Salát (1998). "Optimization of compactin fermentation." *Journal of Industrial Microbiology and Biotechnology* **20** (3-4): 150-152.

Kumar, S., N. Srivastava, B. S. Gupta, R. C. Kuhad and J. Gomes (2014). "Lovastatin production by Aspergillus terreus using lignocellulose biomass in large scale packed bed reactor." *Food and Bioproducts Processing* **92** (4): 416-424.

Lai, L.-S. T., T.-H. Tsai, T. C. Wang and T.-Y. Cheng (2005). "The influence of culturing environments on lovastatin production by Aspergillus terreus in submerged cultures." *Enzyme and Microbial Technology* **36** (5): 737-748.

Liang, B., X. Huang, Y. Teng, Y. Liang, Y. Yang, L. Zheng and X. Lu (2018). "Enhanced Single-Step Bioproduction of the Simvastatin Precursor Monacolin J in an Industrial Strain of Aspergillus terreus by Employing the Evolved Lovastatin Hydrolase." **13** (6): 1800094.

Lin, C.-L., Y.-L. Tang and S.-M. Lin (2011). "Efficient bioconversion of compactin to pravastatin by the quinoline-degrading microorganism Pseudonocardia carboxydivorans isolated from petroleum-contaminated soil." *Bioresource Technology* **102** (22): 10187-10193.

Lin, S.-y., K. Baumann, C. Zhou, W. Zhou, A. E. Cuellar and H. Xue (2021). "Trends in Use and Expenditures for Brand-name Statins After Introduction of Generic Statins in the US, 2002-2018." *JAMA Network Open* **4** (11): e2135371-e2135371.

Liu, C.-L., K. Xue, Y. Yang, X. Liu, Y. Li, T. S. Lee, Z. Bai and T. Tan (2022). "Metabolic engineering strategies for sesquiterpene production in microorganism." *Critical Reviews in Biotechnology***42** (1): 73-92.

Liu, Y., C. Bai, Q. Xu, J. Yu, X. Zhou, Y. Zhang and M. Cai (2018). "Improved methanol-derived lovastatin production through enhancement of the biosynthetic pathway and intracellular lovastatin efflux in methylotrophic yeast." *Bioresources and Bioprocessing***5** (1): 22.

Liu, Y., X. Tu, Q. Xu, C. Bai, C. Kong, Q. Liu, J. Yu, Q. Peng, X. Zhou, Y. Zhang and M. Cai (2018). "Engineered monoculture and co-culture of methylotrophic yeast for de novo production of monacolin J and lovastatin from methanol." *Metabolic Engineering* **45** : 189-199.

Maltsev, Y. I., I. A. Maltseva, S. Y. Maltseva and M. S. Kulikovskiy (2020). "Biotechnological Potential of a New Strain of Bracteacoccus bullatus (Sphaeropleales, Chlorophyta) as a Promising Producer of Omega-6 Polyunsaturated Fatty Acids." *Russian Journal of Plant Physiology* **67** (1): 185-193.

Manzoni, M. and M. Rollini (2002). "Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs." *Applied Microbiology and Biotechnology* **58** (5): 555-564.

McLean, K. J., M. Hans, B. Meijrink, W. B. van Scheppingen, A. Vollebregt, K. L. Tee, J.-M. van der Laan, D. Leys, A. W. Munro and M. A. van den Berg (2015). "Single-step fermentative production of the cholesterol-lowering drug pravastatin via reprogramming of Penicillium chrysogenum." **112** (9): 2847-2852.

Miranda, R. U., L. E. Gómez-Quiroz, M. Mendoza, A. Pérez-Sánchez, F. Fierro and J. Barrios-González (2014). "Reactive oxygen species regulate lovastatin biosynthesis in Aspergillus terreus during submerged and solid-state fermentations." *Fungal Biology* **118** (12): 979-989.

Nielsen, J. and J. T. Pronk (2012). "Metabolic engineering, synthetic biology and systems biology." *FEMS Yeast Research* **12** (2): 103-103.

Novo, M., F. Bigey, E. Beyne, V. Galeote, F. Gavory, S. Mallet, B. Cambon, J.-L. Legras, P. Wincker, S. Casaregola and S. Dequin (2009). "Eukaryote-to-eukaryote gene transfer events revealed by the genome sequence of the wine yeast Saccharomyces cerevisiae EC1118." **106** (38): 16333-16338.

Oliveira, M. C. L. d., A. J. Paulo, C. d. A. Lima, J. L. de Lima Filho, C. M. Souza-Motta, E. E. Vidal, T. P. Nascimento, D. d. A. V. Marques and A. L. F. Porto (2021). "Lovastatin producing by wild strain of Aspergillus terreus isolated from Brazil." *Preparative Biochemistry & Biotechnology* **51** (2): 164-172.

Osmak, M. (2012). "Statins and cancer: Current and future prospects." *Cancer Letters* **324** (1): 1-12.

Panda, B. P., S. Javed and M. Ali (2010). "Optimization of Fermentation Parameters for Higher Lovastatin Production in Red Mold Rice through Co-culture of Monascus purpureus and Monascus ruber." *Food and Bioprocess Technology* **3** (3): 373-378.

Pansuriya, R. C. and R. S. Singhal (2010). "Response surface methodology for optimization of production of lovastatin by solid state fermentation." *Braz J Microbiol* **41** (1): 164-172.

Park, J.-W., J.-K. Lee, T.-J. Kwon, D.-H. Yi, Y.-J. Kim, S.-H. Moon, H.-H. Suh, S.-M. Kang and Y.-I. Park (2003). "Bioconversion of compactin into pravastatin by Streptomyces sp." *Biotechnology Letters***25** (21): 1827-1831.

Pawlak, M. and M. Bizukojc (2013). "Feeding profile is not the sole factor influencing lovastatin production by Aspergillus terreus ATCC20542 in a continuous fed-batch stirred tank bioreactor." *Biochemical Engineering Journal* **81** : 80-89.

Pecyna, M. and M. Bizukojc (2011). "Lovastatin biosynthesis by Aspergillus terreus with the simultaneous use of lactose and glycerol in a discontinuous fed-batch culture." *Journal of Biotechnology***151** (1): 77-86.

Petek, B., M. Villa-Lopez, R. Loera-Valencia, G. Gerenu, B. Winblad, M. G. Kramberger, M.-A.-M. Ismail, M. Eriksdotter and S. Garcia-Ptacek (2018). "Connecting the brain cholesterol and renin–angiotensin systems: potential role of statins and RAS-modifying medications in dementia." *Journal of Internal Medicine* **284** (6): 620-642.

Porcel, E. M. R., J. L. C. López, J. A. S. Pérez and Y. Chisti (2008). "Lovastatin production by Aspergillus terreus in a two-staged feeding operation." **83** (9): 1236-1243.

Porcel, E. R., J. L. C. López, M. A. V. Ferrón, J. A. S. Pérez, J. L. G. Sánchez and Y. Chisti (2006). "Effects of the sporulation conditions on the lovastatin production by Aspergillus terreus." *Bioprocess and*

Biosystems Engineering **29** (1): 1-5.

Rikitake, Y. and J. K. Liao (2005). "Rho GTPases, Statins, and Nitric Oxide." *Circulation Research* **97** (12): 1232-1235.

Ro, D.-K., E. M. Paradise, M. Ouellet, K. J. Fisher, K. L. Newman, J. M. Ndungu, K. A. Ho, R. A. Eachus, T. S. Ham, J. Kirby, M. C. Y. Chang, S. T. Withers, Y. Shiba, R. Sarpong and J. D. Keasling (2006). "Production of the antimalarial drug precursor artemisinic acid in engineered yeast." *Nature* **440** (7086): 940-943.

Rollini, M. and M. Manzoni (2006). "Influence of medium design on lovastatin and mevastatin production byAspergillus terreus strains." *Annals of Microbiology* **56** (1): 47.

Sayyad, S. A., B. P. Panda, S. Javed and M. Ali (2007). "Optimization of nutrient parameters for lovastatin production by Monascus purpureus MTCC 369 under submerged fermentation using response surface methodology." *Applied Microbiology and Biotechnology* **73** (5): 1054-1058.

Shaligram, N. S., S. K. Singh, R. S. Singhal, A. Pandey and G. Szakacs (2008). "Compactin Production Studies Using Penicillium brevicompactum Under Solid-State Fermentation Conditions." *Applied Biochemistry and Biotechnology* **159** (2): 505.

Shaligram, N. S., S. K. Singh, R. S. Singhal, G. Szakacs and A. Pandey (2008). "Compactin production in solid-state fermentation using orthogonal array method by P. brevicompactum." *Biochemical Engineering Journal* **41** (3): 295-300.

Srinivasan, N., K. Thangavelu and S. Uthandi (2022). "Lovastatin production by an oleaginous fungus, Aspergillus terreus KPR12 using sago processing wastewater (SWW)." *Microbial Cell Factories***21** (1): 22.

Sripalakit, P. and A. Saraphanchotiwitthaya (2020). "Lovastatin Production from Aspergillus Terreus ATCC 20542 Under Various Vegetable Oils Used as Sole and Supplementary Carbon Sources." *Pharmaceutical Chemistry Journal* **54** (3): 302-309.

Subhagar, S., R. Aravindan and T. Viruthagiri (2009). "Response surface optimization of mixed substrate solid state fermentation for the production of lovastatin by Monascus purpureus." **9** (4): 303-310.

Suraiya, S., J.-H. Kim, J. Y. Tak, M. P. Siddique, C. J. Young, J. K. Kim and I.-S. Kong (2018). "Influences of fermentation parameters on lovastatin production by Monascus purpureus using Saccharina japonica as solid fermented substrate." *LWT* **92** : 1-9.

Syed, M. B. and M. Rajasimman (2015). "Fermentative production and optimization of mevastatin in submerged fermentation using Aspergillus terreus." *Biotechnology Reports* **6** : 124-128.

Syed, M. B., A. Rajendran, S. Seraman and V. Thangavelu (2014). "Valorization of Agricultural Residues for Compactin Production by Aspergillus terreus MTCC 279 in Mixed Substrate Solid State Fermentation." *Waste and Biomass Valorization* **5** (4): 715-724.

Tartaggia, S., S. Fogal, R. Motterle, C. Ferrari, M. Pontini, R. Aureli and O. De Lucchi (2016). "Chemoenzymatic Synthesis of δ-Keto β-Hydroxy Esters as Useful Intermediates for Preparing Statins."**2016** (19): 3162-3165.

Valera, H. R., J. Gomes, S. Lakshmi, R. Gururaja, S. Suryanarayan and D. Kumar (2005). "Lovastatin production by solid state fermentation using Aspergillus flavipes." *Enzyme and Microbial Technology***37** (5): 521-526.

Vatanparast, M. and Y. Kim (2019). "Yeast engineering to express sex pheromone gland genes of the oriental fruit moth, Grapholita molesta." *Journal of Asia-Pacific Entomology* **22** (3): 645-654.

Xie, X., K. Watanabe, W. A. Wojcicki, C. C. C. Wang and Y. Tang (2006). "Biosynthesis of Lovastatin Analogs with a Broadly Specific Acyltransferase." *Chemistry & Biology* **13** (11): 1161-1169.

Zeng, X., J. Zheng, F. Lu, L. Pan and B. Wang (2022). "Heterologous Synthesis of Monacolin J by Reconstructing Its Biosynthetic Gene Cluster in Aspergillus niger." **8** (4): 407.

Zhou, Z., A. J. Curtis, M. Breslin and M. Nelson (2019). "Letter by Zhou et al Regarding Article, "Statin Toxicity: Mechanistic Insights and Clinical Implications"." *Circulation Research* **124** (12): e120-e120.

Company	Sankyo	Merck	Merck	Sankyo	Sandoz	Pfizer
Half-life	/	3 h	2-3 h	1.5 h	2.3±0.9 h	14 h
Clearance	/	Hepatic	Hepatic	Hepatic and renal	Hepatic	Hepa
Form Administered	/	Inactive lactone	Inactive lactone	Active hydroxy acid	Active hydroxy acid	Activ
Solubility	/	Lipophilic	Lipophilic	Hydrophilic	Lipophilic	Lipop
Derivative	Fungal	Fungal	Fungal	Fungal	Synthetic	Synth
Structure						
Generation	/	First	First	Second	Second	Third
Drug name	Compactin	Lovastatin	Simvastatin	Pravastatin	Fluvastatin	Atorv

Table 2. Submerged fermentation for statins biosynthesis.

Statins	Strain	methods & strategies
Lovastatin	*A. terreus* ATCC 20542	Adding KH_2PO_4, $MgSO_4{\cdot}7H_2O$, NaCl and $ZnSO_4{\cdot}7H_2O$ to the medium
		Adding 2mM or 5mM Zn^{2+} to the medium
		Controlling higher redox potential during fermentation
		Lactose is the initial substrate while feeding with glycerol
		Glycerol is the initial substrate while feeding with lactose
		The age of inoculated spores from 9 days to 16 days
		Carbohydrate concentration of jujube syrup 64 g/ L, yeast extract 15 g/L, PH 6.5
		Adding10μm talc particles to the culture medium
		Soybean and peanut flours as substrate
		The air bubbles diameter of bubble column bioreactor is 0.18cm
		20% dissolved oxygen, 7 days of fermentation at 23
		Adding 50 mg/L tylosin to the culture medium
		Adding 1% w/v coconut oil to the culture medium
	A. terreus URM 5579	60 g/L soluble starch, 15 g/L soybean flour, pH 7.5, 200 rpm and 32 °C for 7 days
	P. ostreatus OBCC 1031	30 g/L glucose, 10 g/L yeast extract, 200 rpm, 28 , and pH 6
	M. purpureus MTCC 369	29.59 g/L glucose, 3.86 g/L NH_4Cl, 1.73 g/L KH_2PO_4, 0.86 g/L $MgSO_4{\cdot}7H_2O$ an
	A. terreus KPR 12	Sago processing wastewater as substrate
	A. terreus GD_{13}	C:N ratio in the culture medium was 37:1; Seven days of fermentation
Compactin	*P.citrinum* NCIM 768	Using chemically-defined medium; Adding surfactant tween 80
		Glc 4.65 g/L, Gly 15.8 g/L, U 0.61 g/L; IA 4.24 days and HT 8.9 days
	A. terreus ATCC 20542	7 days fermentation with modified base medium (CLD); 14 days fermentation wit
	A. terreus	Optimizing the medium constituents like glycerol, $CuCl_2{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$, KH
	P.citrinum MTCC 1256	7.0% glucose, 1.0% yeast extract and 0.1% $MgSO_4$
		Glycerol, peptone, yeast extract, $MgSO_4{\cdot}7H_2O$ and $CaCl_2{\cdot}2H_2O$
	P.citrinum L-18065	10 days of fermentation with 1.5 g/L triton X-100

Table 3. Solid-state fermentation for statins biosynthesis.

Statins	Strain	Description	Titer	Ref.
Lovastatin	*A. flavipes* BICC 5174	Wheat bran as substrates, fermenting in aerated stirred beds for 6 days	16.65 mg/g	(Valera, Gomes et al. 2005)
	O. olearius OBCC 2002	Fermenting of 5 g of barley, 1–2 mm particle diam, at 28 °C	139.47 mg/g	(Atlı, Yamaç et al. 2015)
	M. purpureus MTCC 369	Sago starch as substrate	180.9 mg/g	(Subhagar, Aravindan et al. 2009)
		Long grain rice as substrate	190.2 mg/g	
		Barley as substrate	193.7 mg/g	
	A. terreus MTCC 279	1.5 g of green peas, 1.5 g of millet and 1.5 g of ragi	1467.12 mg/gds	(Syed, Rajendran et al. 2014)
	A. sclerotiorum PSU-RSPG 178	Agricultural wastes as substrates, such as dry corn trunks, rice husks, wild sugarcane and soy bean sludge; adding addition palm oil	0.99 mg/g	(Iewkittayakorn, Kuechoo et al. 2020)
	M. purpureus KCCM 60168	25.64 °C, 14.49 days, 1.32% glucose and 0.20% peptone	13.98 mg/gds	(Suraiya, Kim et al. 2018)
	A. terreus UV 1718	Optimizing medium supplemented with mycological, peptone by response surface methodology	3.723 mg/g	(Pansuriya and Singhal 2010)
	A. terreus PL 10	Controlling packed bed reactor of superficial air velocity at 23.37 cm/min; 54% substrate composition	1.86 mg/g	(Kumar, Srivastava et al. 2014)

Statins	Strain	Description	Titer	Ref.
	M. purpureus MTCC 369 and *M. ruber* MTCC 1880	Co-culturing *m. purpureus* MTCC 369 and *m. ruber* MTCC 1880 for 14 days	2.83 mg/g	(Panda, Javed et al. 2010)
Compactin	*P. brevicompactum* WA 2315	Adding various supplements (glycerin, etc.); optimize supplement pH=7.5	0.815 mg/gds	(Shaligram, Singh et al. 2008)
		Control the substrate initial water content 50%; fermenting 168 hours; adding glycerol	1.25 mg/gds	(Shaligram, Singh et al. 2008)
	A. terreus MTCC 279	Using 1.5 g of green peas, 1.5 g of millet and 1.5 g of ragi as combinations of the substrates	389.34 mg/gds	(Syed, Rajendran et al. 2014)
	A. terreus GCBL-03	Bagasse as substrates, using potassium hydroxide to pretreat bagasse	30.63±1.24 mg/100mL	(Javed, Bukhari et al. 2016)

Table 4. Engineering strains strategies to improve the production of statins.

Statins	Strain	Description
Simvastatin	*S. cerevisiae* BY4741	Introducing the synthetic gene of monacline J; adding the acyl-donor dimethylbu
Lovastatin	*P. pastoris* GS115	Introducing lovastatin synthesis gene into *P. pastoris* GS115; using dihydromona
		Overexpressing the statins pump protein TapA (a membrane protein that enables
	A. terreus ATCC 20542	Inserting the antibiotic marker hygromycin B (*hyg*) within the *gedC* gene that en
	A. terreus EM_{19}	Inducing *A. terreus* GD_{13} for three cycles
	Fusarium sp. Alaa-20	Enhancing mutagenesis induction and three successive gene recombination of *Fus*
Pravastatin	*Streptomyces sp.* Y-110	Adding compactin to the medium intermittently
	P. chrysogenum DS50662	Introducing the compactin pathway into the beta-lactam-negative *P. chrysogenu*