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Enhancement of Lovastatin Production by Supplementing Polyketide Antibiotics to the Submerged Culture of *Aspergillus terreus*

Zhihua Jia · Xiaoli Zhang · Yaling Zhao · Xuejun Cao

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Abstract Feedback inhibition existed in lovastatin biosynthesis from *Aspergillus terreus*. Exogenous lovastatin and other different polyketide antibiotics biosynthesized by polyketide synthase were supplemented to the cultures of *A. terreus* to investigate their influences on lovastatin production. Supplementing exogenous lovastatin of 100 mg l^{-1} at the early stage of fermentation and the fast stage of its biosynthesis resulted in decreases of 76.4% and 20% in final lovastatin production, respectively. However, the fungal cell growth was not affected; the growing cycle was only prolonged in the submerged cultivation. Separate supplementation of the five kinds of polyketide antibiotics such as tylosin, erythromycin, tetracycline, daunorobin, and rifamycin to the cultures resulted in increases of about 20~25% in the final lovastatin production. Especially, supplementing tylosin of 50 mg l^{-1} at the beginning of lovastatin biosynthesis led to the final lovastatin production of 952.7±24.3 mg l^{-1}, which was improved by 42% and 22% compared with that produced in the control and the original culture, respectively. These results are helpful to understand the regulations on lovastatin biosynthesis and improve the final desired metabolite contents in many antibiotics production.

Keywords *Aspergillus terreus* · Lovastatin · Production · Polyketide antibiotics · Tylosin

Introduction

Lovastatin is one of the competitive inhibitors of enzyme hydroxymethyglutaryl coenzyme A reductase (HMG-CoA), which catalyzes the rate-limiting step in cholesterol biosynthesis,

Z. Jia
College of Life Sciences, Northwest A&F University, 22 Xinong Road, Yangling, Shaanxi 712100, China

Z. Jia · X. Cao (✉)
State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology,
130 Meilong Road, Shanghai 200237, China
e-mail: caoxj@ecust.edu.cn

X. Zhang (✉) · Y. Zhao
College of Chemical Engineering, Northwest University, 229 North Taibai Road, Xi'an 710069, China
e-mail: xlzhang@nwu.edu.cn

resulting in lowered blood cholestol [1, 2]. It can be produced by submerged cultures of *Penicillium* species [3], *Aspergillus terreus* [2], and *Monascus* species [4] via the polyketide synthase (PKSs), which are responsible for synthesizing many secondary metabolites with complex chemical structures.

The biosynthesis of lovastatin is coordinated by two separate PKS systems possessing a large range of biosynthetic activities [5]. The dihydromonacolin L synthase consists of at least two enzymes, the iterative LovB (also known as lovastatin nonaketide synthase) and LovC, a putative enoyl reductase. These two enzymes appear to be all that is necessary for the 35 separate reactions postulated to be necessary for the biosynthesis of dihydromonacolin L from acetyl-coenzyme A, malonyl-coenzyme A, NADPH, and S-adenosylmethionine (SAM). Nonaketide monacolin J, the immediate biosynthetic precursor of lovastatin, is assembled by post-PKS modification of dihydromonacolin L with the CYP450 oxygenases LovA. The other immediate precursor 2-methylbutyric acid is synthesized by LovF (also known as lovastatin diketide synthase) through a single condensation between an acetyl-CoA and a malonyl-CoA. These two precursors are combined at the C8 hydroxyl group of monacolin J to yield lovastatin catalyzed by a 46-kDa acyltransferase, LovD (Fig. 1) [6–8]. Inactivation of any enzymes in the pathway would lead to the reduction of final lovastatin production [9].

It has been identified that a feedback regulation exists in lovastatin biosynthesis from *A. terreus*, i.e., lovastatin inhibits its own biosynthesis. Release of this feedback inhibition may improve the lovastatin production markedly. However, the regulatory mechanism is still not clear [10]. In order to improve the lovastatin production by using the strategy that the feedback regulation occurring in its own biosynthetic pathway is released or reduced, it is necessary to understand the detailed information of this regulatory mechanism. In the improvement programs of many antibiotic-producing mutants, selection of streptomycin-, gentamicin-, or paromomycin-resistant mutants has been successfully used to increase production of many unrelated antibiotics [11]. Moreover, during the investigation of feedback regulation on polyketide metabolism in tylosin biosynthesis, exogenous addition of glycosylated tylosin precursors or other macrolides antibiotics such as rosaramicin and spiramycin can stimulate the final production of tylosin in the cultivation of *Streptomyces fradiae* [12, 13]. Although the regulatory mechanism is still unclear, it does not affect the application for the production of antibiotics originated from other bacteria or fungi, and for the selection of mutants resistant to the antibiotic itself or the structurally related metabolite. Previously, there is no special report on applying this idea and strategy to the production of lovastatin.

In this study, exogenous addition of lovastatin to the submerged culture of *A. terreus* was used to investigate the influences on cell growth, lovastatin production, and the feedback regulation on its own biosynthesis. Simultaneously, any other polyketide antibiotics such as tylosin, erythromycin, daunorobin, tetracycline, and rifamycin were selected to investigate their influences on lovastatin biosynthesis in submerged culture of *A. terreus*. These five antibiotics are all biosynthesized by PKSs in which polyketide biosynthesis proceeds by simple, repetitive condensations of acetate or propionate monomers in a manner that closely parallels fatty acid synthesis [14]. Structural complexity is introduced by variation in the stereochemistry and the degree of reduction after each condensation as well as by downstream enzymes that catalyze cyclizations, oxidations, alkylations, glycosylations, and other transformations. They probably regulate the biosynthesis of lovastatin. This idea and the addition strategy may be helpful to the hyper-production of lovastatin or many other antibiotics from bacteria and filamentous fungi.

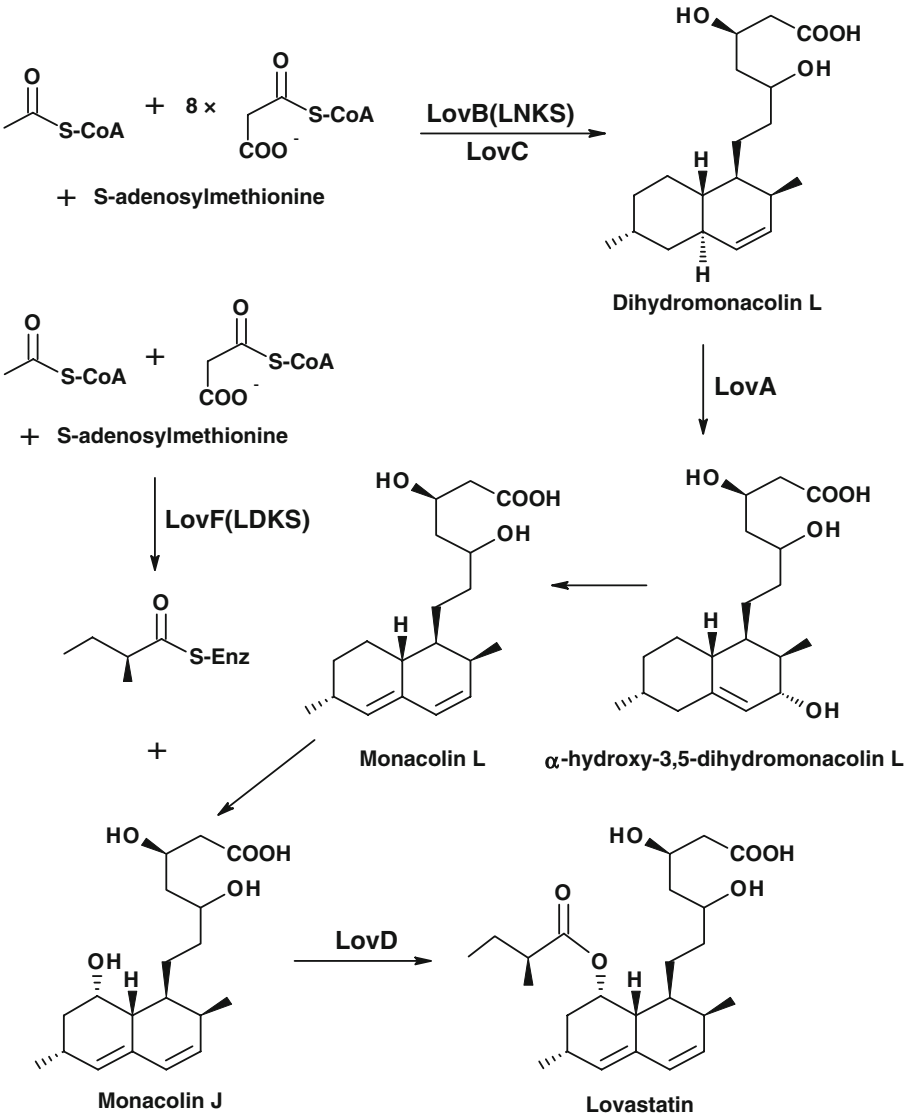

Fig. 1 Biosynthesis of lovastatin

Materials and Methods

Chemicals

Lovastatin lactone was gifted by Dr. X.B. Mao from Chong-qing Academy of Chinese Materia Medica. Tylosin and Daunorobin were donated by Xi'an Hengtong Guanghua Pharmaceutical Co., Ltd. (China) and Xi`an Libang Pharmaceutical Co., Ltd. (China), respectively. Tetracycline, erythromycin, and rifamycin were purchased from Xi'an Lijun Pharmaceutical Co., Ltd. (China).

Microorganism, Media, and Culture Conditions

Strain improvement was initiated on *A. terreus* ATCC 20542 by random mutation. Spores of *A. terreus* ATCC 20542 were exposed to UV (15 W, 254 nm, 25 cm) for 4-5 min. Then, the spores were diluted and poured plates. The plates were covered in black polythene bags and incubated at 28 °C for 5 days. Primary screening of the strains were carried out using a novel agar plug method [15]. This method utilizes the anti-fungal property of lovastatin to produce a zone of inhibition against *Neurospora crassa*. Strains with improved yields were further subjected to aforementioned treatments. The high-yielding strain was designated as LA414, and all subsequent experiments were carried out using *A. terreus* LA414. The slants and plates medium all contained 20 g l^{-1} of glucose, 20 g l^{-1} of malt extract, 1 g l^{-1} of peptone, and 20 g l^{-1} of agar. After being inoculated from the original slant, the inoculated slants were incubated at 28 °C for 5 days and then used for inoculation of seed culture. Conidiospores were harvested by washing the slant cultures with sterile distilled water, filtrated, centrifuged (~4,000 rpm, 5 min), and resuspended in sterile distilled water. After being counted with a hemacytometer, the spore suspension was diluted to about 5×10^7 spore ml^{-1} for inoculation.

The seed culture medium consisted of the following components: tomato paste, 40 g l^{-1}; oat meal, 10 g l^{-1}; glucose, 10 g l^{-1}; corn steep liquor, 5 g l^{-1}; trace element solution, 10 ml l^{-1} with pH of 6.8 before sterilization. The trace element solution contained (g l^{-1}): $FeSO_4{\cdot}7H_2O$ 1, $MnSO_4{\cdot}4H_2O$ 1, $CuCl_2{\cdot}2H_2O$ 0.025, $CaCl_2{\cdot}2H_2O$ 0.1, H_3BO_3 0.056, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.01, and $ZnSO_4{\cdot}7H_2O$ 0.2. Two milliliters of the above spore resuspensions (approximate 10^8 spores) were inoculated to a 100-ml shake flask containing 20 ml of seed culture medium and incubated at 28 °C on a rotary shaker (220 rpm) for 24 h.

The production culture was conducted in a 100-ml conical flask containing 20 ml of the production medium. Two milliliters of seed culture (10%) was inoculated. All fermentations were carried out at 28 °C on a rotary shaker (220 rpm) for 8 days. Multiple flasks were run at the same time to ensure reproducibility. Based on some publications [16–18], an optimal production medium for lovastatin production by this mutant was screened in our preliminary work. The production medium contained 67.56 g l^{-1} of soluble starch (3% of carbon content), 10 g l^{-1} of yeast extract powder, 2.5 g l^{-1} of polyethylene glycol 2000, 2 g l^{-1} of KH_2PO_4, and 1 g l^{-1} of $(NH_4)_2SO_4$. The original pH of the medium was adjusted to 6.5 before sterilization.

Experimental Design

To investigate the feedback inhibitory mechanism of lovastatin on its own biosynthesis in submerged culture of *A. terreus*, the exogenous sterilized β-hydroxy acid form of lovastatin (mevinolinic acid) was supplemented to the inoculated cultures at the beginning of the culture and at the period of lovastatin biosynthesis (the third day), respectively. The final concentration of exogenous mevinolinic acid in the cultures was 100 mg l^{-1}. No additional mevinolinic acid was supplemented to the control culture. Dry cell weight and lovastatin production were determined at an interval of 2 days.

The other five antibiotics biosynthesized by polyketide synthase pathway including tylosin, erythromycin, daunorobin, tetracycline, and rifamycin were selected to investigate their effects on lovastatin biosynthesis. After being sterilized by an ultrafiltration membrane, the sodium salt solutions of these antibiotics were supplemented to the inoculated cultures at the early stage of fermentation. The final concentrations of antibiotics were all 50 mg l^{-1}. No antibiotics were supplemented to the control culture.

Different strategies of antibiotics supplementation including supplementation concentration and supplementation time were adopted to probe into the regulation of polyketide antibiotics on lovastatin biosynthesis. Therefore, different initial levels of tylosin were supplemented to the inoculated cultures of *A. terreus* at the early stage of shake flask culture. The final supplementation concentrations of tylosin in the cultures were 20, 50, 100, and 200 mg l^{-1}, respectively. No tylosin was supplemented to the control culture.

To investigate the effects of supplementation time of polyketide antibiotics on lovastatin biosynthesis, the sterilized tylosin was supplemented to the inoculated cultures at days 0, 2, 4, and 6, respectively. The final concentration of supplemental tylosin in the cultures was 50 mg l^{-1}.

Analytical Methods

The biomass concentration was measured by filtering a culture broth through preweighed filter paper, and the cake was washed with distilled water and then dried at 60°C for sufficient time until constant weight. For sampling, three identical flasks were taken each time.

The culture broths were extracted by using ethyl acetate. Thin layer chromatography (TLC) was used for initial screening the monacolin J (R_f=0.25), 2-methylbutyric acid (R_f=0.8), lovastatin lactone (R_f=0.6), and its hydroxyl acid (R_f=0.5) using dichloromethane/acetic acid (85:15, *v*/*v*) as the developing solvent (Fig. 2) [19]. The desired products content was determined by HPLC using a Kromasil C18 (4×150 mm, 4 μm particle sizes) of Eliter Company (Liaoning, China) column with an eluent comprising methanol/0.1% aqueous phosphoric acid (77.5:22.5, *v*/*v*) [20]. The injection volume was 5 μl. Elution was performed at a flow rate of 1 ml min^{-1} at 25°C. A tunable absorbance detector with UV wavelength of 238 nm was used for monitoring eluate. Samples were centrifuged at 5,000 rpm for 10 min before injection. The final lovastatin product was detected as the sum of these two forms.

The known standard samples of lovastatin and the intermediate monacolin J were applied for comparison qualitatively and quantitatively. The β-hydroxyl acid of lovastatin was prepared by dissolving its lactone form in the mixture of 0.1 M NaOH and ethanol (1:1,*v*/*v*), then heating at 50°C for 20 min and neutralizing with 1 M HCl [19]. Monacolin J was prepared by refluxing lovastatin acid with 1 M LiOH overnight [21]. The reaction was cooled to room temperature, after that acidified with 2 M HCl (pH<2), and extracted with CH_2Cl_2. The organic extracts were dried by Na_2SO_4, filtered, and evaporated. The residue was dissolved in toluene and reflux with a CaH_2 Soxhlet for 1 h. After cooling to room temperature, the solvent was evaporated, and the residue was fractionated by flash column chromatography (40% EtOAc in hexanes) to obtain the pure monacolin J.

Results

Feedback Inhibition in Lovastatin Biosynthesis

It is well known that feedback inhibition of desired metabolites on their own biosynthesis occurs in many antibiotics productions [22]. Supplementation of exogenous lovastatin to the culture of *A. terreus* would inhibit its own biosynthesis to a certain extent. Increase of additional lovastatin level would lag the startup of its own biosynthesis; however, it did not affect the cell growth [10]. The effects of supplementing exogenous lovastatin on its own biosynthesis and cell growth were investigated in the submerged culture of *A. terreus*.

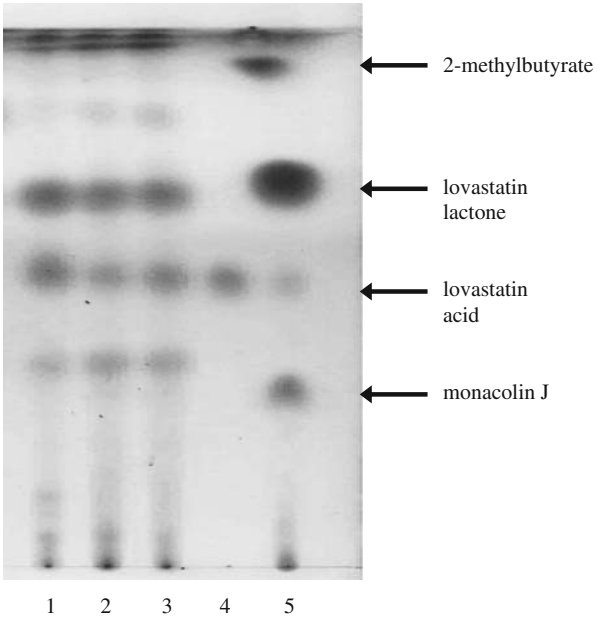

Fig. 2 TLC of extracts from *A. terreus* mutant strain. *Lanes 1 to 3* extracts from strain LA414, *lane 4* standard lovastatin acid, *lane 5* standard hydrolyzed mixture of lovastatin

Compared with the control culture, supplementing exogenous lovastatin of 100 mg l^{-1} at the beginning of cultivation did lag the cell growth a little (Fig. 3). At the late stage of exponential growth (the third day), 24.45±0.26 g dry cell weight (DCW) l^{-1} of biomass was obtained from the culture with supplementing exogenous lovastatin, while 26.7± 0.36 gDCW l^{-1} of biomass was attained from the control run. A decrease of 8.4% was thus produced. The final biomass was basically identical, 29.9±0.31 and 29.15±0.37 gDCW l^{-1}, respectively. When supplementing the same quantum of exogenous lovastatin at the period of its biosythesis, the final biomass was 30.15±0.42 gDCW l^{-1}. These results indicated that excess of lovastatin will lag the cell growth to a certain extent but does not influence the final growth.

The pH of the broth also varied correspondingly (Fig. 3). When supplementing exogenous lovastatin to the cultures at the early stage of fermentation, the pH of broth quickly declined to 3.70±0.13, while pH of 4.41±0.08 was obtained in the control at the early stage of exponential growth (the third day). Thereafter, both of them tardily appeared to be invariable. A constant pH value of 3.1 was reached at the end of cultivation. However, the pH of broth in the culture with supplementing exogenous lovastatin at the third day decreased more slowly than that in the control. The final pH value of the former broth was 3.34±0.02.

Although supplementation of exogenous lovastatin did not affect the fungal cell growth, but only prolonged the growing cycle, its own biosynthesis was strongly restrained (Fig. 3). Supplementation of exogenous lovastatin at the early stage of cultivation did markedly inhibit its final biosynthesis. Only a final lovastatin concentration of 162.5±22.9 mg l^{-1} was attained, while 687.5±24.5 mg l^{-1} was obtained in the control. A decrease of 76.4% was thus produced. However, final lovastatin of 546.0±25.2 mg l^{-1} was achieved in the case of supplementing exogenous lovastatin at the third day. The production was reduced by 20% compared with the control.

López reported that supplementation of exogenous lovastatin to the cultures after inoculation with spores for 24 h would result in decreases of final lovastatin production and

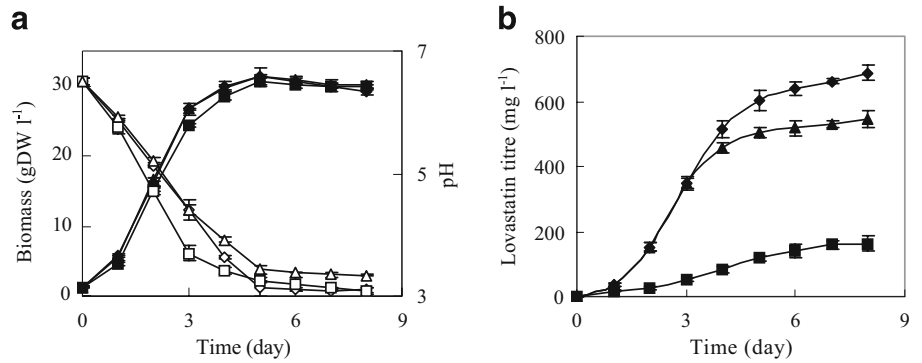

Fig. 3 Effects of additional lovastatin on cell growth (**a**) and its own production (**b**) in submerged cultures of *A. terreus*. Control (*closed diamonds*), adding at day 0 (*closed squares*), adding at day 3 (*closed triangles*) and pH (*opened* symbols)

productivity in submerged cultivation of *A. terreus* ATCC20542. The startup of its own biosynthesis was also prolonged. Rise of exogenous lovastatin level from 50 to 100 and 250 mg l^{-1} could accelerate to decrease the final lovastatin production in cultures. The final lovastatin production and productivity were decreased for 7%, 36%, and 73% compared with the control run, respectively [10].

The effects of adding levels of exogenous lovastatin on *A. terreus* ATCC20542 and its mutant LA414 were compared in Table 1. The mutant was more sensitive to lovastatin formation than the parent strain. The former was easier to appear feedback inhibition than the latter. Therefore, it is more convenient to screen better lovastatin-resistant mutant or to research the feedback regulatory mechanism in lovastatin biosynthesis.

Comparison of Various Polyketides Biosynthesized by PKS Pathway

In the investigation of the feedback regulation on lovastatin biosynthesis, the other five polyketide antibiotics such as tylosin, erythromycin, daunorobin, tetracycline, and rifamycin were also researched. These five antibiotics were supplemented to the liquid medium at the early stage of fermentation (day 0) with 50 mg l^{-1} of initial concentration. The cultures were sampled and analyzed for cell growth and lovastatin production as showed in Table 2.

Table 1 Effects of spiked lovastatin on its own biosynthesis in submerged cultures of *A. terreus*.

Strain	Spiked lovastatin (mg l^{-1})	Relative production (%)	
LA414	0	100	In this study
	50	25	
	100	24	
	100 (day 3)	80	
ATCC 20542	0	100	[13]
	50	75	
	100	46	
	250	18	

Table 2 Effects of various kinds of spiked polyketides on cell growth and lovastatin biosynthesis in submerged cultures of *A. terreus*.

	Spiked concentration (mg l^{-1})	Biomass (gDCW l^{-1})	Lovastatin titre (mg l^{-1})	Relative production (%)
Control	0	29.52±0.45[a]	652.3±23.4	100
Lovastatin	50	29.55±0.35	162.4±12.6	25
Tylosin	50	30.45±0.35	786.9±13.2	121
Erythromycin	50	30.55±0.15	763.1±25.6	117
Daunoribin	50	29.45±0.30	805.0±37.0	123
Tetracycline	50	30.35±0.25	772.0±28.0	118
Rifamycin	50	30.95±0.05	813.0±14.0	125

Exogenous polyketides antibiotics were supplemented to the cultures at the early stage of fermentation

[a] The maximum errors were calculated from three independent samples

Compared with the control run, the exogenous polyketide antibiotics did not affect the cell growth. The net lovastatin yields on biomass obtained from the cultures with the above five exogenous antibiotics were 25.8±0.4, 25.0±0.8, 27.3±1.3, 25.4±0.9, and 26.3±0.5 mg $gDCW^{-1}$, respectively, while the yield obtained from the control run was 22.1±0.8 mg $gDCW^{-1}$. Except for decrease of 75% (only 5.5±0.4 mg $gDCW^{-1}$) in the case of supplementing exogenous lovastatin, other five polyketide antibiotics all improved the net yields of final lovastatin for approximate 20~25% compared with the control.

During the fermentations, fewer accumulations of the two intermediate precursors in lovastatin biosynthesis, monacolin J and 2-methylbutyric acid, were detected in all cultures (Table 3). Figure 1 shows that LovD protein catalyzes transferring of 2-methylbutyric acid to monacolin J to form lovastatin. Thus, the percent conversions of these two intermediate metabolites (x_{monJ} and x_{MBA}) were calculated (Table 3), respectively. Compared with the control run, the formation of total monacolin J and its percent conversion in the cultures were decreased for 74.5% and 2.5% in the presence of exogenous lovastatin, respectively. Simultaneously, these two values of 2-methylburyric acid were decreased for 56.8% and

Table 3 Effects of supplementing various kinds of polyketide antibiotics on accumulations of the key intermediate precursors in lovastatin biosynthesis.

	$C_{mon\ J}$ (mg l^{-1})	x_{monJ} (%)[a]	C_{MBA} (mg l^{-1})	x_{MBA} (%)[b]
Control	43.6±6.4[c]	92.2	38.5±4.7	81.1
Lovastatin	14.5±2.3	89.9	46.8±6.7	46.7
Tylosin	82.6±7.8	88.3	65.6±8.2	75.2
Erythromycin	77.2±6.6	88.7	58.9±7.5	76.6
Daunorobin	86.5±8.3	88.1	66.7±6.8	75.3
Tetracycline	78.7±5.9	88.6	56.8±5.6	77.4
Rifamycin	90.8±8.8	87.6	64.4±7.9	76.1

[a] The molar ratio between the converted and the total monacolin J (monJ), the latter including both the converted and the accumulated monacolin J in the process

[b] The molar ratio between the converted and the total 2-methylbutyric acid (MBA), the latter including both the converted and the accumulated 2-methylbutyric acid in the process

[c] The maximum errors were calculated from three independent samples

42%, respectively. However, supplementation of other five polyketide antibiotics to the cultures would improve the formations of the two precursors markedly. Nevertheless, their percent conversion were all decreased for 5~6%.

Effect of Tylosin Supplementation Concentration

Various levels of tylosin including 20, 50, 100, and 200 mg l^{-1} were supplemented to the fermentation medium at the first stage of cultivation, respectively. The biomass and lovastatin production were determined as showed in Fig. 4. The highest biomass of 29.73±1.63 g l^{-1} was obtained in the case of supplementing a lower level of tylosin (50 mg l^{-1}), which resulted in an increase of 7.5% compared with the control. However, higher levels of exogenous tylosin (beyond 50 mg l^{-1}) had no noticeable effect on cell growth. When supplementing 20, 50, 100, and 200 mg l^{-1} of exogenous tylosin, the corresponding lovastatin productions obtained were 675.0±25.0, 792.4±34.1, 626.7±18.6, and 556.8±22.5 mg l^{-1}, respectively. The highest lovastatin production was obtained with supplementing tylosin of 50 mg l^{-1}. The final production was 20% higher than that obtained in the control culture. Only lovastatin of 660.3±15.2 mg l^{-1} was reached in the latter. The lovastatin production was gradually repressed with the levels of tylosin increasing. The corresponding net yields of lovastatin on biomass were 24.1±0.9, 26.7±1.2, 22.5±0.7, and 19.8±0.8 mg $gDCW^{-1}$, respectively. The net yield on biomass of 23.8±0.5 mg $gDCW^{-1}$ was obtained in the control run. These suggest that lower levels of exogenous tylosin probably promote the lovastatin biosynthesis to a certain extent. When the level is beyond 50 mg l^{-1}, lovastatin biosynthesis will be suppressed.

Effects of Tylosin Supplementation Time

The optimal supplementation time of tylosin for cell growth and lovastatin production was also investigated in submerged cultivation of *A. terreus*. Exogenous tylosin of 50 mg l^{-1} was supplemented to the cultures at days 0, 2, 4, and 6, respectively. The final biomass and lovastatin production were determined on the eighth day. The results are showed in Fig. 5. These results suggest that the supplementation time of tylosin influenced the cell growth. The highest biomass of 29.73±1.63 gDCW l^{-1} was obtained when the supplementation of tylosin was at the early stage of cultivation. With the extension of the supplementation time, the cell growth declined gradually. The final biomass was only 27.33±0.13 gDCW l^{-1} when

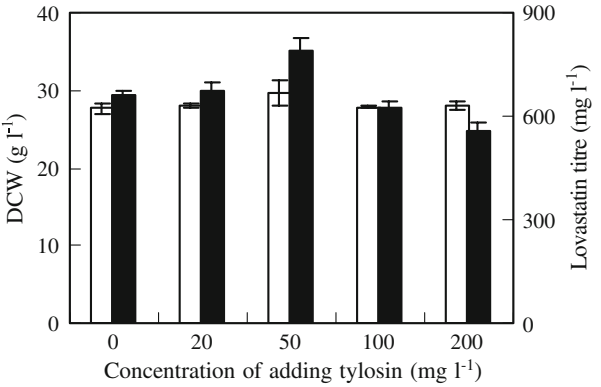

Fig. 4 Effects of levels of additional tylosin on cell growth (*open bars*) and lovastatin production (*closed bars*) in submerged cultures of *A. terreus*

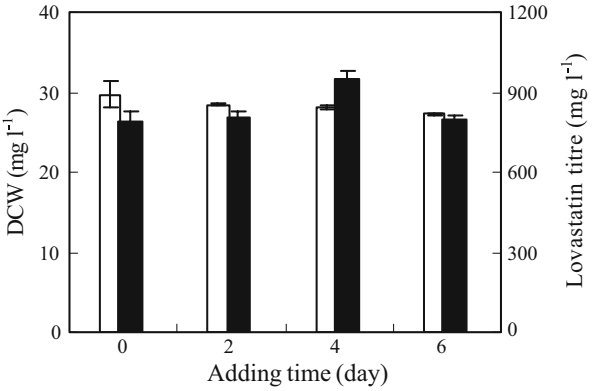

Fig. 5 Effects of supplementing time of additional tylosin on cell growth (*open bars*) and lovastatin production (*closed bars*) in submerged cultures of *A. terreus*

the supplementation of tylosin was at the sixth day of cultivation, which was decreased by about 8% from the case when supplementation was at the beginning.

Apparently, supplementing exogenous tylosin at different periods markedly regulated the biosynthesis of lovastatin. The final lovastatin production obtained from the control run reached to 668.8±18.8 mg l^{-1}, and its corresponding net yield on biomass was 24.2± 0.7 mg $gDCW^{-1}$. Supplementation of 50 mg l^{-1} of exogenous tylosin to the cultures at days 0, 2, 4, and 6 produced 782.3±28.1, 806.2±23.6, 952.7±24.3, and 797.3±15.8 mg l^{-1} of final lovastatin production, respectively. The corresponding net yields of lovastatin on biomass were 26.7±1.1, 28.4±0.8, 33.9±0.9, and 29.2±0.6 mg $gDCW^{-1}$, respectively. The highest lovastatin production and its net yield on biomass were attained when the supplementation of tylosin was at the fourth day of cultivation. It was the rapid formation period of lovastatin. Compared with the control run, an increase of 42% was produced. This is also an increment of 22% relative to that supplementing at the early stage of fermentation. Regardless of cell growth, the net yields of lovastatin on biomass increased by 40% and 27%, respectively.

Discussion

During the rapid growing phase, the pH of cultures varied with the active metabolism of the cells. Supplementation of exogenous lovastatin might have postponed the cell growth to a certain extent, which led to the response of cells to this phenomenon and accelerated the cell metabolism. After cell growth entering into the stationary phase, i.e., the fast production phase of desired metabolites, the fungal cell might have adapted to the response and gradually came back to the normal status. The pH of broth became constant. In the case of supplementing exogenous lovastatin at the production phase, the cell physiological activity mostly advanced to the regulation of secondary metabolism due to the feedback regulation of lovastatin on its own biosynthesis. Deceleration of the primary metabolism probably caused less accumulation of organic acids, thus the pH of broth descended slower than that of the control. Lai et al. [23] reported that another key metabolite of *A. terreus*, itaconic acid, was formed during the course of lovastatin production simultaneously. Accumulation of the former metabolite would reduce the latter's production. The biosynthesis of the former metabolite was identified that it was originated from the Embden-Meyerhof-Parnas pathway and the tricarboxylic acid cycle [24]. Therefore, the accumulation of organic

acid intermediates (citric acid, pyruvic acid, and acetic acid) might occur very easily during the biosynthetic pathway of itaconic acid, which resulted in decrease of the broth pH.

López et al. reported that supplementing exogenous lovastatin to the cultures may delay the startup of its own biosynthesis [10]. In this study, supplementation of exogenous lovastatin at the early stage of fermentation did remarkably restrain the activity of certain or some key enzymes involved in lovastatin biosynthesis. These enzymes possibly participated in regulating on some primary metabolism of its initial biosynthetic precursors. The biosynthesis of lovastatin was thus hindered. However, supplementation of exogenous lovastatin at the beginning of its biosynthesis would partially lessen the inhibition. It is suggested that the feedback regulation on lovastatin biosynthesis did not delay the startup but restrained the biosynthesis. Understanding well the lovastatin biosynthetic pathway and the related genes reveals that self-resistance to lovastatin is provided by ORF1 or *lvrA* gene [5]. The former encodes an esterase-like protein, while the latter encodes a protein very much like known HMG-CoA reductases. Introduction of *lvrA* into the lovastatin-sensitive *Aspergillus nidulans* confers high-level lovastatin resistance [7].

According to the analysis of the percent conversion of monacolin J and 2-methylbutyric acid in the cultures, exogenous lovastatin would inhibit the biosynthesis of these two intermediate metabolites. However, the inhibition to the former was more remarkable than to the latter. These results indicate that the feedback inhibition of lovastatin on its own biosynthesis is independent on LovD protein. Furthermore, it is suggested that it is not only regulated by the self-resistant ORF1 or *lvrA* gene but also related to the inhibition of a certain enzyme (LovA or LovC) that is involved in the formation of monacolin J. The other five polyketide antibiotics stimulate the biosynthesis of lovastatin to some extent, probably due to a similar action mechanism. This stimulation probably occurs in the period of precursor formation or in the early stage of lovastatin biosynthesis. However, the presence of exogenous polyketide antibiotics resulted in appreciable decreases of the percent conversions of the key intermediate metabolites. The activity of LovD is thereby inhibited to some extent. Supplementing tylosin at the rapid formation stage of lovastatin was more propitious to the latter's biosynthesis. The polyketide antibiotics such as tylosin possibly play a role of effector in regulation of lovastatin biosynthesis. More detailed information of the stimulation mechanism should be researched in the future. These results are helpful to understand the regulation of lovastatin biosynthesis and to improve the final desired metabolite content in many antibiotics productions.

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