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Induction of a High-Yield Lovastatin Mutant of *Aspergillus terreus* by $^{12}C^{6+}$ Heavy-Ion Beam Irradiation and the Influence of Culture Conditions on Lovastatin Production Under Submerged Fermentation

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Abstract Heavy-ion beams, possessing a wide mutation spectrum and increased mutation frequency, have been used effectively as a breeding method. In this study, the heavy-ion beams generated by the Heavy-Ion Research Facility in Lanzhou were used to mutagenize *Aspergillus terreus* CA99 for screening high-yield lovastatin strains. Furthermore, the main growth conditions as well as the influences of carbon and nitrogen sources on the growth and the lovastatin production of the mutant and the original strains were investigated comparatively. The spores of *A. terreus* CA99 were irradiated by 15, 20, 25, and 30 Gy of 80 MeV/u $^{12}C^{6+}$ heavy-ion beams. Based on the lovastatin contents in the fermentation broth, a strain designated as *A. terreus* Z15-7 has been selected from the clone irradiated by the heavy-ion beam. When compared with the original strain, the content of lovastatin in the fermentation broth of *A. terreus* Z15-7 increased 4-fold. Moreover, *A. terreus* Z15-7 efficiently used the carbon and nitrogen sources for the growth and production of lovastatin when compared to the original strain. The maximum yield of lovastatin, 916.7 μg/ml, was obtained as *A. terreus* Z15-7 was submerged cultured in the chemically defined medium supplemented with 3% glycerol as a carbon source, 1% corn meal as an organic nitrogen source, and 0.2% sodium nitrate as an inorganic nitrogen source at 30 °C in the shake flask. The result shows that heavy-ion beam irradiation is an effective method for the mutation breeding of lovastatin production of *A. terreus*.

Keywords *Aspergillus terreus* · Heavy-ion beam · Mutation breeding · Lovastatin

Introduction

Breeding technology using mutations induced by ion beam irradiation has undergone major developments and has been applied in various fields. High linear energy transfer (LET)

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radiation (e.g., ion particles) causes localized, dense ionization within the cell compared to low-LET radiation (e.g., X-rays or γ-rays). Heavy-ion beams, possessing the higher LET and distinct biological effects than low-LET radiations (e.g., X-rays and γ-rays), have a wide mutation spectrum and increased mutation frequency and have been used effectively as a breeding method [1]. Although the mechanisms of heavy-ion-induced mutation are not well clarified at the molecular level, many studies have revealed great biological effects caused by heavy-ion irradiation at the cellular and molecular levels. For example, heavy-ion irradiation induces chromosome breaks including chromatid discontinuity; misalignment of the distal, chromatid ring; chromosomal aberrations; and irreparable DNA breaks in mammalian and human cells [1] as well as induces developmental abnormalities, chromosome rearrangement, breaks and aberrations, and increased mutation rates in plant cells [1, 2]. *Arabidopsis thaliana* analysis demonstrated that ion particles induced different kinds of mutations, such as inversions, translocations, short deletions, rearrangement, and point-like mutation, and could therefore produce various types of mutants [3]. Heavy ion beams induce changes in miRNA expression levels and the specificity miRNAs in rice [4]. Carbon ions generated more than ten times the number of mutations induced by γ-rays and caused base changes, including transversions (G:C→T:A, 68.7%), transitions (G:C→A:T, 13.7%), and deletions/insertions (17.6%) in budding yeast strain [5]. Recently, heavy ion beams have been used as a novel and efficient method for tumor radiotherapy and mutagenesis in plant breeding [1]. However, there are less reports of heavy-ion beam irradiation used for breeding microbe strains, although more than 30 microbe strains have been treated by ion implantation. These strains can produce various fermentation products, including enzymes, vitamins, organic acids, amino acids, and antibiotics [6].

Lovastatin, a kind of fungal metabolite, acts as a competitive inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the rate-limiting enzyme of cholesterol biosynthesis [7, 8]. It can effectively reduce plasma cholesterol levels in various mammalian species, including humans, and is thereby effective in the therapy of hypercholesterolemia [9]. Lovastatin was the first hypocholesterolemic drug to be approved by the United States Food and Drug Administration [10]. In addition, these compounds can prevent stroke and reduce the development of peripheral vascular disease. Statin therapy has biological effects beyond the level of cholesterol reduction, including atheromatous plaque stabilization, modification of the atherosclerosis progression, improved endothelial functions, modulation of inflammatory responses, and prevention of thrombus formation [11]. Lovastatin can be produced as a secondary metabolite by a variety of filamentous fungi, including *Penicillium* sp. [12], *Monascus ruber* [7], and *Aspergillus terreus* [8]. Commercial production of lovastatin uses *A. terreus* submerged fermentation, which has been extensively investigated [11]. Improving the productivity of *A. terreus* is essential for the commercial success of a lovastatin fermentation process. A number of studies have attempted to increase lovastatin production through more efficient processes; most of these studies have focused on the optimization of *A. terreus* cultivation [13–18].

It is well known that the culture medium has a significant influence on the biosynthesis of lovastatin and its rate of production. Selection and composition optimization of an optimum medium is therefore important for establishing a process for producing lovastatin. Carbon and nitrogen sources and C/N ratio play a dominant role in fermentation productivity because these nutrients are directly linked with the formation of the biomass and the metabolite [14–18].

Although mutation breeding is an effective method of improving the production of lovastatin, and many mutagenic techniques have been used to improve the productivity of *A. terreus*, to our knowledge, there have been no reports of using heavy-ion irradiation to induce high-yield lovastatin mutants of *A. terreus* [6]. The objective of this study was to

examine the mutagenic effect of heavy-ion beam irradiation on *A. terreus* and to obtain the high-yield lovastatin strains of *A. terreus*. Furthermore, we investigated the main growth conditions and the influences of carbon and nitrogen sources on the growth and lovastatin production of *A. terreus* mutants relative to the original strain.

Materials and Methods

Strain, Media, and Growth Conditions

A. terreus CA99 used in the experiments was obtained from China Agricultural University, incubated at 30 °C for 3–5 days on malt extract agar slants, and then stored at 4 °C. Seed cultures were prepared by transferring a loopful of spores from the malt extract agar slants into a 100-ml conical flask containing 50 ml basal medium (50 g glucose, 10 g corn meal, 5 g peptone, 2 g NaCl, 0.5 g $MgSO_4 \cdot 7H_2O$, and 0.5 g KH_2PO_4 in 1,000 ml of distilled water, adjusted to pH 6.0), then incubated at 30 °C for 24 h in a rotary shaker at 150 rpm.

The basal medium was used in the experiments. For tests of the effect of carbon sources on the growth and lovastatin production of the strains, 5% of each carbon source and 1% corn meal were used in the chemically defined medium (i.e., 2 g NaCl, 0.5 g $MgSO_4 \cdot 7H_2O$, and 0.5 g KH_2PO_4 in 1,000 ml of distilled water, adjusted to pH 6.0). To test the effects of nitrogen sources on the growth and lovastatin production of the strains, 1% of each nitrogen source and 5% glycerol were used in the chemically defined medium.

Heavy-Ion Irradiation

The National Laboratory of Heavy-Ion Accelerator, Lanzhou, was established in 1991. There are a series of ion-beam acceleration facilities intensively used in the past two decades, such as the Heavy-Ion Research Facility in Lanzhou (HIRFL). The heavy-ion beam irradiation was conducted at the HIRFL [1]. *A. terreus* CA99 was grown for 4 days at 30 °C on the malt extract agar slants, and then the spores were harvested by adding 5 ml of sterile water and rubbing the slant surface with a pipette to release the spores. The suspension was transferred to a sterile conical flask containing glass beads to disrupt the clumps of spores and oscillated for 5 min. Microscopy demonstrated that the spores were dispersed and diluted to a concentration of 10^8 spores per milliliter. A volume of 2 ml of the spore suspension was pipetted into a 30-mm Petri dish and irradiated by $^{12}C^{6+}$ heavy-ion beam. The carbon ion energy was 80.55 MeV/u, and the dose rate was 4 Gy/min. The irradiating doses of the heavy-ion beam were 15, 20, 25, and 30 Gy.

Mutant Screening

After the heavy-ion irradiation, the spore suspension was diluted to 10^{-4}, 10^{-5}, and 10^{-6}, then 100 μl of the dilution was pipetted into 90 mm Petri dishes containing malt extract agar plates and spread uniformly using a spreader. The plates were incubated at 30 °C in an incubator. After 5 days, the mutants were primarily selected based on colony morphology and the growth rate of a single colony on the plates. For rescreening, 5 ml of the seed cultures of the mutants was transferred to a 100-ml conical flask containing 50 ml of medium (50 ml glycerol, 10 g corn meal, 2 g NaCl, 0.5 g $MgSO_4 \cdot 7H_2O$, and 0.5 g KH_2PO_4 in 1,000 ml of distilled water, adjusted to pH 6.0) and incubated at 28 °C in a rotary shaker at 150 rpm for 15 days; then, the contents of lovastatin in the fermentation broth were

measured. The high-yield lovastatin strains were selected and maintained on malt extract agar slants at 4 °C for further experiments.

Analytical Methods

The lovastatin in the fermentation broth was extracted with ethyl acetate [13]. After 15 days of liquid culture, the fermentation broth was adjusted to pH 3.0 using HCl, after which an equal volume of ethyl acetate was then added. After shaking at 180 rpm for 12 h at ambient temperature, the fermentation broth and the mycelium pellets were filtrated through pre-weighed membrane filters and the residual mass was washed three times with ethyl acetate. The biomass was determined by gravimetric analysis after the mycelium pellets were dried at 65 °C to a constant weight. The organic and aqueous phases from the filtrate were separated in a separating funnel. The organic phase was dried in a rotavapor under vacuum at 45 °C. The dried residue was dissolved in a little acetone and mixed with 1 g of silica gel; then, the mixture was added in a chromatography column and eluted with the eluent of petroleum ether and acetone (7:1, v/v). After detection using thin-layer chromatography, the eluent containing lovastatin was collected and dried in a rotavapor under vacuum at 45 °C. The dried residue was dissolved in 5 ml of 75% ethanol and used to determine the lovastatin content.

Lovastatin contents were determined by the dual-wavelength UV spectrophotometry method [19, 20]. A lovastatin standard was acquired from the National Institute for the Control of Pharmaceutic and Biological Products. For the construction of a calibration curve, 64 μg/ml of the lovastatin standard solution was prepared by dissolving 3.2 mg of the lovastatin standard in 50 ml of 75% ethanol, then 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 ml of the standard solution were diluted to a constant volume of 10 ml and the absorbance at 246 nm and 254 nm were measured using a UV759S spectrometer (Shanghai Precision & Scientific Instrument Co. Ltd., China). The absorbance difference between 246 nm and 254 nm, which is the characteristic absorption peak of lovastatin, was calculated, and the results were used to plot a calibration curve. The linear regression equation was $y = 0.0276X + 0.0054(R^2\ 0.9994)$. It indicated that a good linear relationship appeared between the concentration of lovastatin in the range of 3.2–57.6 μg/ml and the absorbance difference between 246 nm and 254 nm.

Statistical Analyses

Data presented are the means of at least three independent experiments and expressed as the mean±SE. Data were analyzed using by one-way ANOVA with post hoc tests. Comparisons between the mean values were made by the least significant difference (LSD test) at $P < 0.05$, and standard error (SE) was calculated. The statistical analyses were performed using SPSS/PC software ver. 14.0 (SPSS Inc., Chicago, IL, USA).

Results

Effect of Heavy-Ion Irradiation on *A. terreus* CA99

The 80 MeV/u $^{12}C^{6+}$ heavy-ion irradiation had a marked influence on the survival of *A. terreus* CA99 spores. The survival rate of spores decreased sharply when the heavy-ion irradiation dose increased from 15 to 30 Gy. The lethality rate of spores approached 100% as heavy-ion irradiation dose reached 30 Gy (Table 1). After primary screening, the

Table 1 Effect of the heavy-ion irradiation on the spore survival of *A. terreus* CA99

Dose (Gy)	Colony count (cfu)	Lethality rate (%)
0	90,000	0
15	10,000	88.9
20	353	99.6
25	218	99.8
30	11	99.9

lovastatin productivity of 42 strains irradiated by heavy-ion beams was detected. The lovastatin yields of nine strains were increased, 29 strains were decreased, and four strains were unchanged. Based on the lovastatin productivity, the positive mutation rate was 0.09% and the negative mutation rate was 0.29%.

Screening of the Mutants for Lovastatin Production

After the heavy-ion irradiation, the spore suspension was diluted and spread uniformly on the malt extract agar plates. The plates were incubated at 28 °C for 5 days. Thirteen strains with faster growth among the 10,582 single colonies were selected for rescreening. The mutant strains were submerged cultured in a shake flask at 28 °C in a rotary shaker at 150 rpm for 15 days, and then the contents of lovastatin in the fermentation broth were determined. Among the 42 strains, the mutant designated *A. terreus* Z15-7 was selected as a target strain for further experiments, as its lovastatin production (689.1 μg/ml) was increased by 4-fold relative to the original strain (171.2 μg/ml) (Fig. 1a).

Time Course of Lovastatin Production of *A. terreus* CA99 and *A. terreus* Z15-7

To investigate the time course of lovastatin production of *A. terreus* Z15-7, submerged fermentation was performed using the essential nutrients medium at 28 °C and shaken at 150 rpm for 15 days. Both the lovastatin yields and the biomasses of *A. terreus* Z15-7 (mutant) and *A. terreus* CA99 (original strain) were determined. The results are illustrated in Fig. 1a. The curves show that the accumulation of lovastatin increased quickly after 9 days of cultivation. The maximum content of lovastatin, 689.1 μg/ml, appeared on the 15th day in *A. terreus* Z15-7 cultures, with a 403% increase from the *A. terreus* CA99 culture content (171.2 μg/ml). The growth curve profile of *A. terreus* Z15-7 was similar to that of *A. terreus* CA99 except the growth rate was faster than that of *A. terreus* CA99 (Fig. 1b).

Genetic Stability of Lovastatin Production of *A. terreus* Z15-7

To examine the genetic stability of *A. terreus* Z15-7, the strain was continuously subcultured to the ninth generation, with the lovastatin measured after each generation, indicating that the lovastatin yields of *A. terreus* Z15-7 remained steady after being subcultured for nine generations (Table 2).

Effects of Temperature, Carbon Sources, and Nitrogen Sources on Lovastatin Production of *A. terreus* Z15-7

To investigate the effects of the substrates on the growth and the lovastatin production of *A. terreus* Z15-7, a defined medium with a sole carbon source and a sole nitrogen source was

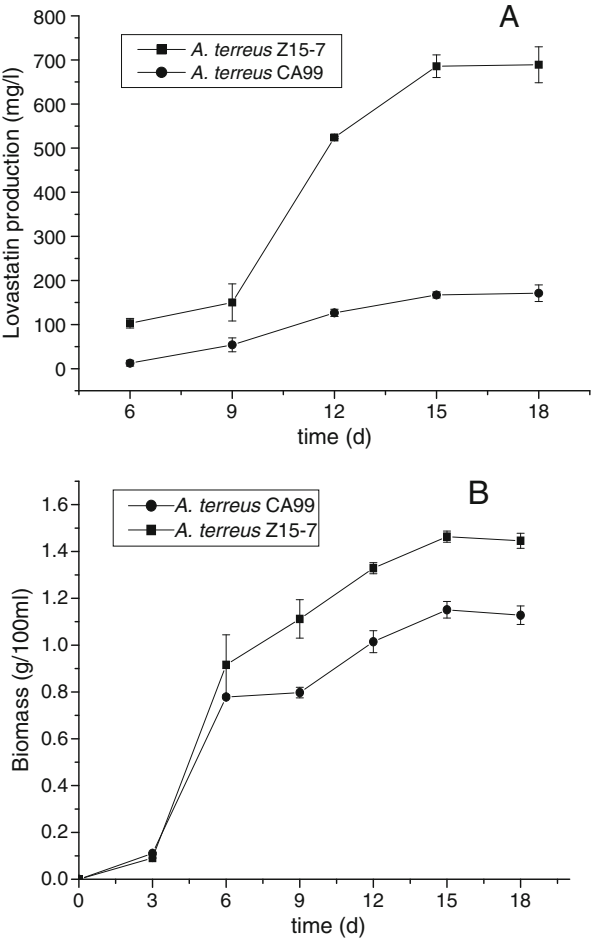


Fig. 1 **a** Kinetics profiles of the lovastatin production of *A. terreus* Z15-7 and *A. terreus* CA99 in submerged fermentation. **b** Growth curves of *A. terreus* Z15-7 and *A. terreus* CA99

developed. Several inorganic and organic nitrogen sources were tested in the shake flask cultures using the defined medium. When growth media contained 5% glucose, glycerol, or soluble starch as a sole carbon source combined with 1% corn meal, the biomasses of Z15-7 cultured in all three kinds of carbon source media were markedly higher than that of CA99. The greatest biomasses of Z15-7 and CA99 were obtained in the test with 5% soluble starch, and the second greatest was that with 5% glucose. The lovastatin yields of Z15-7 cultured in all three

Table 2 Genetic stability of *A. terreus* Z15-7

Generations	The 15-day production of lovastatin (mg/l)	Maintain rate of lovastatin (%)
0	842.5±61.5	100
3	833.2±48.5	98.89
6	826.2±59.4	98.06
9	809.8±81.8	96.12

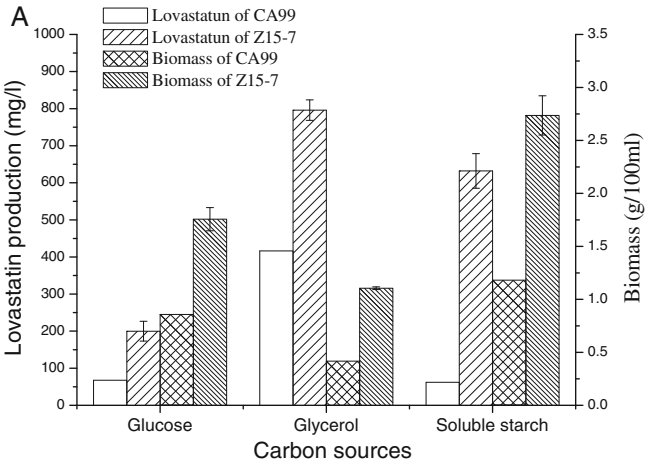
kinds of carbon source media were markedly higher than those of CA99. The highest lovastatin yield of Z15-7 was obtained in the test with 5% glycerol and the next was that with 5% soluble starch. These results showed that the optimum carbon source for the strain growth and biomass production was soluble starch; however, for the lovastatin production the optimum carbon source was glycerol (Fig. 2a). When growth media contained peptone, yeast extract, or corn meal as the sole organic nitrogen source and 5% glycerol as the carbon source, yeast extract significantly increased the growth of Z15-7 and CA99. However, the greatest yield of lovastatin appeared in the test with corn meal, suggesting that corn meal may be the optimum organic nitrogen source for the lovastatin production of Z15-7 (Fig. 2b), although it is generally regarded as a carbon source. When KNO_3, NH_4NO_3, or $NaNO_3$ as the sole inorganic nitrogen source and 5% glycerol as a carbon source were added in the media, all three nitrogen sources significantly improved the growth of Z15-7 and the highest biomass appeared in the test with NH_4NO_3. However, $NaNO_3$ significantly improved the lovastatin production of Z15-7, suggesting that $NaNO_3$ was the optimum inorganic nitrogen source for the lovastatin production of Z15-7 (Fig. 2c).

The effects of glycerol on the growth and the lovastatin production of *A. terreus* Z15-7 were further investigated. The strain was submerged cultured in a defined medium supplemented with 1% to 11% glycerol in shake flask cultures. The results showed that the concentrations of glycerol significantly influence the growth and lovastatin production, and a dose-dependent effect was investigated in the tests. The greatest biomass was measured in the test with 9% of glycerol; however, the highest lovastatin yield, 916.7 μg/ml, appeared in the test with 3% of glycerol (Fig. 3a). This finding suggests that glycerol is a crucial carbon source for the synthesis of lovastatin and 3% of glycerol is a suitable concentration for the growth and the lovastatin production of *A. terreus* Z15-7.

To examine the optimum temperature for lovastatin production of *A. terreus* Z15-7, the strain was submerged cultured in conical flasks containing essential nutrients medium at different temperatures, i.e., 25, 28, and 30 °C, and the variable temperature (first 3 days at 30 °C and then at 25 °C) in a rotary shaker at 150 rpm for 15 days. The results showed that the biomasses of *A. terreus* Z15-7 cultured at 28 °C, 30 °C, or the variable temperature had no significant difference from each other but were significantly higher than those cultured at 25 °C. Interestingly, the lovastatin yield of *A. terreus* Z15-7 cultured at 30 °C was significantly higher than those cultured at 25 °C, 28 °C, or the variable temperature (Fig. 4), indicating that different optimum temperatures were needed for the growth and lovastatin production of *A. terreus* Z15-7.

Qualitative Analysis of Lovastatin Produced by *A. terreus* Z15-7

It is necessary to confirm whether lovastatin was synthesized by *A. terreus* Z15-7. We examined the UV and IR spectra of the sample purified from fermentation broth and the lovastatin standard. Figure 5 shows that the three UV characteristic absorption peaks (230 nm, 238 nm, and 246 nm) of the sample in 75% ethanol are the same as the data of 229 nm, 237 nm, and 246 nm [4]. The IR spectra of the sample and the lovastatin standard were recorded in potassium bromide disks at room temperature by a Vertex 70/70v FT-IR spectrometer (Bruker Co., Germany). Figure 6 shows that the same IR spectra appeared in the sample (i.e., 3,539.98, 2,928.53, 1,700.37, and 1,219.63 cm^{-1}) and the lovastatin standard (i.e., 3,540.63, 2,965.37, 1,699.64, and 1,220.01 cm^{-1}). Therefore, the results indicated that lovastatin was synthesized by *A. terreus* Z15-7 and its fermentation broth contains lovastatin.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Biomass of CA99
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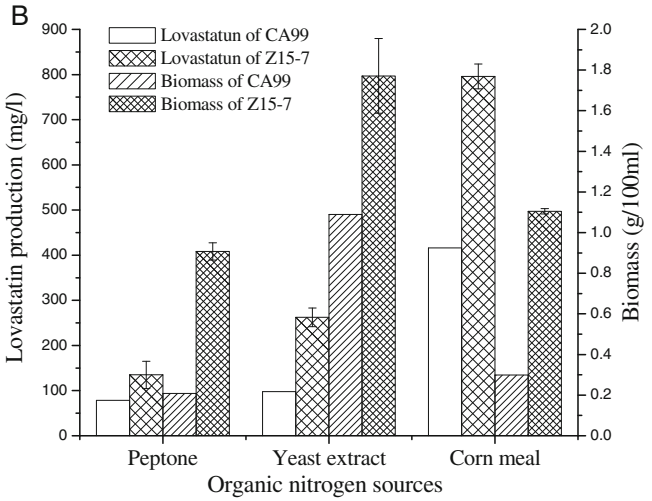
Lovastatin production (mg/l)
Biomass (g/100ml)
Glucose
Glycerol
Soluble starch
Carbon sources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Lovastatin production (mg/l)
Biomass (g/100ml)
Peptone
Yeast extract
Corn meal
Organic nitrogen sources

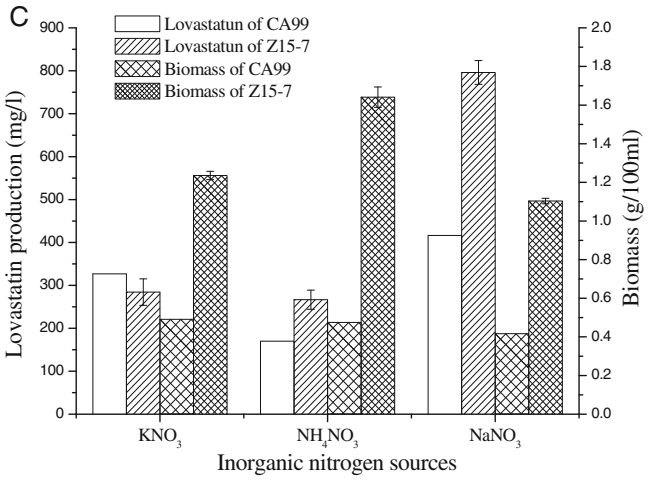

C
Lovastatun of CA99
Lovastatun of Z15-7
Biomass of CA99
Biomass of Z15-7
Lovastatin production (mg/l)
Biomass (g/100ml)
KNO3
NH4NO3
NaNO3
Inorganic nitrogen sources

Fig. 2 Effects of carbon sources and nitrogen sources on the lovastatin production of *A. terreus* Z15-7 and *A. terreus* CA99. **a** Carbon sources were added at 5%; **b** organic nitrogen sources were added at 1%; **c** inorganic nitrogen sources were added at 0.2%. Submerged fermentation was conducted in a 250-ml conical flask containing 100 ml of fermentation medium designed for lovastatin production in a rotary shaker for 15 days at 28 °C and at 150 rpm

Discussion

Although more than 30 microbe strains, which can accumulate various fermentation products, including enzymes, vitamins, organic acids, amino acids, and antibiotics, have been treated by ion implantation [6], there are few reports that highlight heavy-ion irradiation mutagenesis breeding in microbes. To date, heavy-ion irradiation has not been applied to the mutation breeding and lovastatin production activity of *A. terreus*. In this experiment, 15 to 30 Gy of 80 MeV/u $^{12}C^{6+}$ heavy-ion beam irradiation was used to irradiate the spores of *A. terreus* CA99. Some mutant strains having a faster growth rate and a higher yield of lovastatin production compared with the original strain were screened. Further screening selected the mutant designated *A. terreus* Z15-7, which can produce four times the lovastatin than that of the original strain under the optimized culture conditions. We demonstrated that heavy-ion beam irradiation had a prominent mutagenic effect on *A. terreus* and strongly reduced the spores survival of *A. terreus*. These findings suggest that it is a feasible method for the mutation breeding of lovastatin production of *A. terreus*.

The production of lovastatin of *A. terreus* was greatly influenced by the cultivation conditions and the medium constituents. A number of studies have investigated the enhancement of lovastatin on the laboratorial and industrial scales. These include studies on the effects of different fermentation parameters, such as carbon and nitrogen sources [14, 21]. In addition to relief of glucose repression, carbon source starvation is required, while

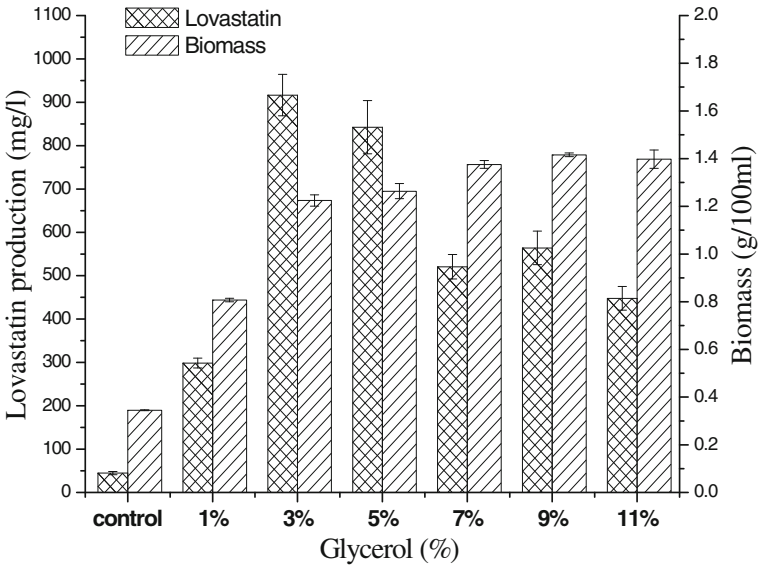


Fig. 3 Effects of glycerol on the growth and lovastatin production of *A. terreus* Z15-7. Submerged fermentation was conducted in a 250-ml conical flask containing 100 ml of fermentation medium designed for lovastatin production in a shaker for 15 days at 28 °C and at 150 rpm

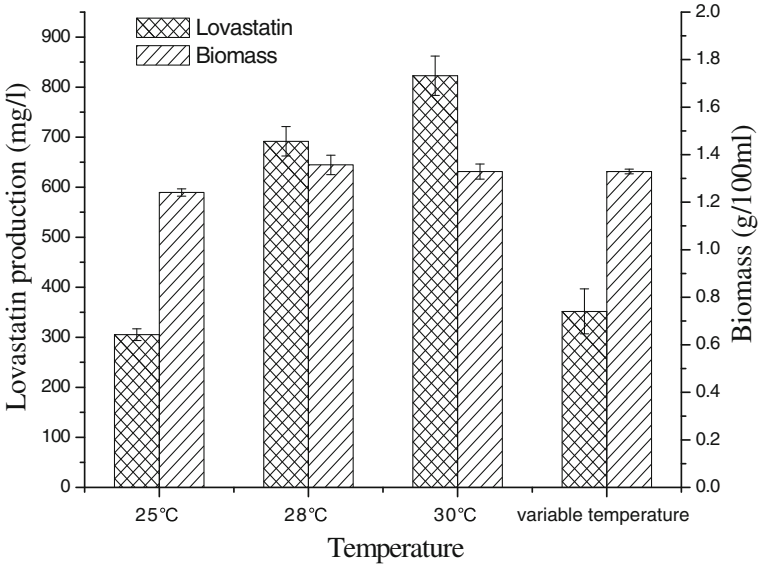
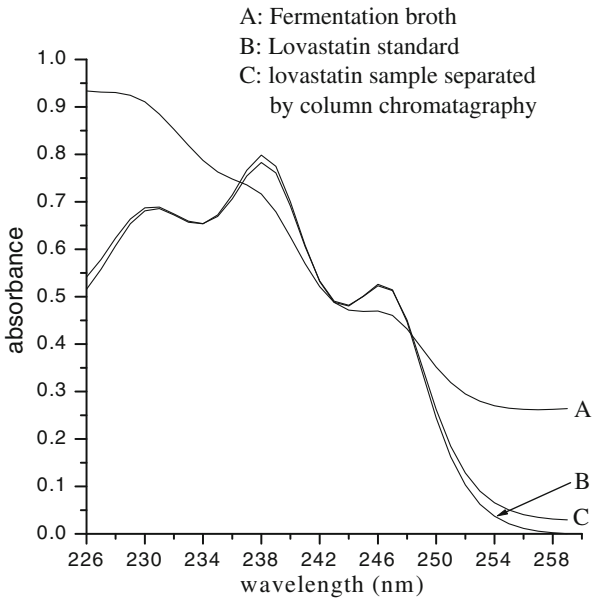


Fig. 4 Effect of temperature on the growth and lovastatin production of *A. terreus* Z15-7. Submerged fermentation was conducted in a 250-ml conical flask containing 100 ml of fermentation medium designed for lovastatin production in a shaker for 15 days at 28 °C and at 150 rpm

glutamate did not repress biosynthesis. A higher specific productivity was obtained with the defined medium on glucose and glutamate [17], and with a slowly metabolized carbon source under conditions of nitrogen limitation [14]. A combination of both a rapidly and a slowly metabolized sugar may be beneficial to lovastatin production by *A. terreus* [17]. The

Fig. 5 UV spectrum of lovastatin sample (in 75% ethanol)



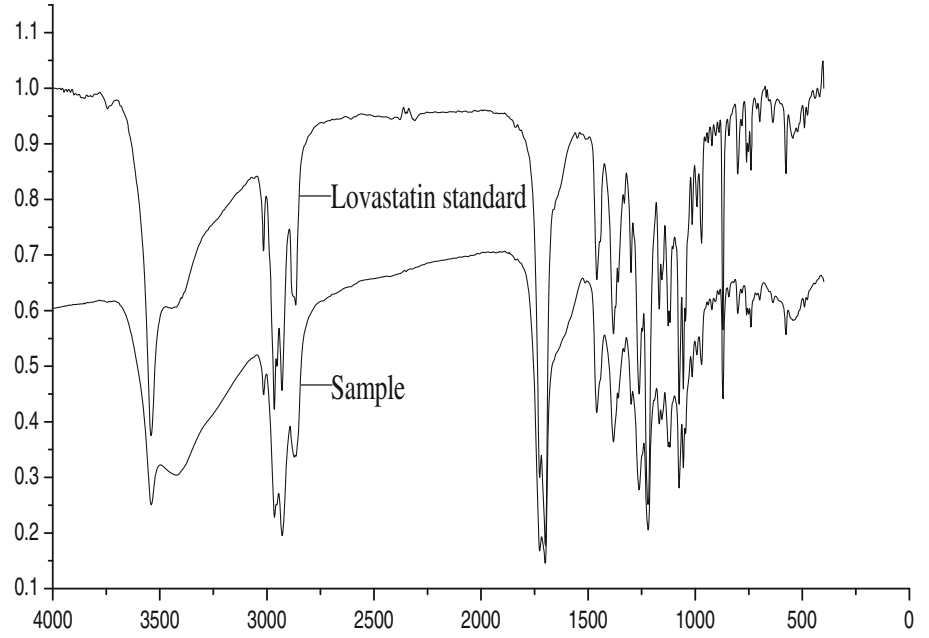


Fig. 6 IR spectrum of lovastatin sample and lovastatin standard (KBr)

slowly utilized carbon source was consumed only after glucose exhaustion [14]. Lactose was the best carbon source for lovastatin biosynthesis and more propitious to the release of catabolite repression [17, 18]. Glycerol was the most beneficial to lovastatin production because it is introduced into the glycolysis pathway for absolute oxidation and it decreased the effect of catabolite repression in lovastatin biosynthesis [18]. Moreover, in glycerol metabolism, NADH can be generated, which is required for the formation of 4,5-dihydromonacolin L catalyzed by nonaketide synthase (LNKS) during the biosynthesis of lovastatin [16]. Soluble starch as the sole carbon source resulted in the highest biomass and the second highest lovastatin titer. These results were consistent with the report by Jia et al. [18]. The present study certified that the optimum carbon source for the lovastatin production of *A. terreus* Z15-7 was glycerol.

The type of the nitrogen source affects the production of lovastatin of *A. terreus*. Glutamate and histidine as the single organic nitrogen source gave the highest lovastatin biosynthesis level [17] and yeast extract, soybean meal [14], and peptone [15] are the preferred complex nitrogen sources when compared to corn steep liquor. The optimal initial C/N mass ratio for attaining high productivity of lovastatin was 40 [14]. In these studies, corn steep liquor was used as a nitrogen source in the medium. In our study, the highest lovastatin production was obtained when corn meal was used as nitrogen source and glycerol used as carbon source. The effect of an inorganic nitrogen resource on the lovastatin production was less clear. Our experiment showed that $NaNO_3$ was the most beneficial inorganic nitrogen source to lovastatin production.

It is known that temperature greatly influences the growth of different fungi and production of secondary metabolites [22]. The cultivation temperature is another main factor influencing lovastatin production. Many studies confirmed that 28 °C is the optimum temperature for the growth and lovastatin production of *A. terreus* [13, 17, 23], whereas the

highest lovastatin production of *A. terreus* Z15-7 was obtained at 30 °C with the medium of optimization, suggesting that the maximum temperature for lovastatin production of *A. terreus* Z15-7 was at 30 °C.

In addition, the dual-wavelength UV spectrophotometry method was used to measure the concentration of lovastatin in this study. Although HPLC is a precise method for the detection of lovastatin in many studies, our experiment proved that UV spectrophotometry is also a simple, inexpensive, reliable, and suitable method for the quantification of lovastatin in the fermentation broth [20]. This method was also used to detect most plasma amino acids by Palego [24] and can be used for therapeutic drug monitoring. Its application to other body fluids, tissue, and cell preparations is expected.

In conclusion, this study screened a steady heredity mutant strain of *A. terreus*, *A. terreus* Z15-7, by heavy-ion beam irradiation. Relative to the original strain, the lovastatin yield of *A. terreus* Z15-7 increased 4-fold. Furthermore, the optimum culture temperature and carbon and nitrogen sources for the growth and the lovastatin production were also altered in *A. terreus* Z15-7. The results showed that the maximum lovastatin yield, 916.7 μg/ml, was obtained in the chemically defined medium supplemented with 3% glycerol as a carbon source, 1% corn meal as an organic nitrogen source, and 0.2% sodium nitrate as an inorganic nitrogen source at 30 °C in the shake flask cultures. Similar results were reported by Lai et al. [15] and Jia et al. [18]. Lovastatin production of 1.05 g/l was obtained by a highly productive mutant of *A. terreus* ATCC 20542 at the following (g/l): lactose (89.5), glycerol (30.2), peptone (6.0), soybean meal (4.2), glucose (20.0), and corn steep liquor (10.0), under response surface methodology [15]. The highest lovastatin titer (937.5± 12.5 mg/l) was produced by a mutant strain of *A. terreus* LA414 at 51.2 g/l of glycerol and 10 g/l of yeast extract powder [18].

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