J Ind Microbiol Biotechnol (2004) 31: 48–50
DOI 10.1007/s10295-004-0108-y

SHORT COMMUNICATION

J. L. Casas López · E. M. Rodríguez Porcel
M. A. Vilches Ferrón · J. A. Sánchez Pérez
J. M. Fernández Sevilla · Y. Chisti

Lovastatin inhibits its own synthesis in *Aspergillus terreus*

Received: 30 September 2003 / Accepted: 10 December 2003 / Published online: 23 January 2004

Abstract Lovastatin suppresses its own synthesis in the microfungus *Aspergillus terreus*. The inhibitory effect was documented by spiking identical batch cultures with pure lovastatin (0, 50, 100 and 250 mg/l) 24 h after initiation from spores.

Keywords Lovastatin · *Aspergillus terreus* · Product inhibition

Introduction

Statins are a group of drugs that are used for lowering blood cholesterol. Lovastatin ($C_{24}H_{36}O_5$, Mevinolin, Monacolin K and Mevacor) is a widely used natural statin produced by various fungi [1, 3, 4, 6]. It is also the precursor of the other available semisynthetic statins.

Lovastatin is produced commercially using batch fermentations of *Aspergillus terreus*. Such fermentations usually last for less than 10 days. Fed-batch fermentations of *A. terreus* have been investigated for producing lovastatin and are said to be superior to batch cultures [7, 8, 9, 11, 13]. Since lovastatin is a secondary metabolite, earlier studies focused on the effects of the composition of the culture medium and the fermentation conditions on its production [2, 7, 8, 9,11, 13]. However, there is no discussion in the literature of how the concentration of lovastatin might affect its own synthesis by *A. terreus*. Product inhibition of fermentation is a crucial influence in many industrial processes [12], but has not been documented for lovastatin production.

The present work reports on the inhibitory effect of lovastatin on its own synthesis by *A. terreus*. Lovastatin production by identical cultures that had been spiked with pure lovastatin (0, 50, 100 and 250 mg/l) 24 h after initiation from spores was monitored to prove the inhibitory effect.

J. L. Casas López (✉) · E. M. Rodríguez Porcel
M. A. Vilches Ferrón · J. A. Sánchez Pérez
J. M. Fernández Sevilla
Department of Chemical Engineering,
University of Almería, 04120 Almería, Spain
E-mail: jlcasas@ual.es
Tel.: +34-950-015314
Fax: +34 950-015484

Y. Chisti
Institute of Technology and Engineering,
Massey University, Private Bag 11 222,
Palmerston North, New Zealand

Materials and methods

Microorganism and inoculation

Aspergillus terreus ATCC 20542 was obtained from the American Type Culture Collection. The fungus was maintained in Petri dishes of PDA (potato dextrose agar). After inoculation from the original slant, the dishes were incubated at 28 °C for 5 days and subsequently stored at 5 °C. A suspension of spores was obtained by washing the Petri dish cultures with a sterile aqueous solution of 2% Tween 20. The resulting suspension was centrifuged (~2,800 *g*, 5 min) and the solids were resuspended in sterile distilled water. The spore concentration was determined spectrophotometrically at 360 nm. A standard curve was used to correlate the optical density to direct spore counts that had been carried out with a flow cytometer (Coulter Epics XL-MCL).

Culture conditions

Cultures were incubated at 28 °C on a rotary platform shaker (150 rpm, 2.6 cm stroke) in 250-ml shake-flasks filled with 100 ml of the medium. Flasks without cotton closures were placed in a sterile-air growth chamber. Air was introduced into the chamber through a 0.2-μm sterile Millipore filter at a flow rate high enough to provide a constant gas composition within the chamber. The medium contained (per liter): 114.26 g lactose, 5.41 g soybean meal, 0.8 g KH_2PO_4, 0.4 g NaCl, 0.52 g $MgSO_4{\cdot}7H_2O$, 1 mg $ZnSO_4{\cdot}H_2O$, 2 mg $Fe(NO_3)_3{\cdot}9H_2O$, 0.04 mg biotin and 1 ml of a trace element solution. The trace element solution contained (per liter): 100 mg $Na_2B_4O_7{\cdot}10H_2O$, 50 mg $MnCl_2{\cdot}4H_2O$, 50 mg $Na_2MoO_4{\cdot}2H_2O$, and 250 mg $CuSO_4{\cdot}5H_2O$. The pH of the medium was adjusted to 6.5 with NaOH (0.1 N) before sterilization. The flasks were inoculated with 450 μl of a spore suspension that had been standardized to contain $1.9{\times}10^8$ spores/ml. In order to avoid any negative effects of lovastatin on spore germination, a

β-hydroxyacid form of lovastatin was added 24 h after inoculation in order to obtain the desired final concentration. The incubation lasted up to 7 days.

Analytical methods

Biomass

The biomass (as dry weight) was determined by filtering a known volume of the broth through a 0.45-μm Millipore cellulose filter, washing the cells with sterile distilled water, and freeze-drying the solids.

Lovastatin

Lovastatin was measured in its β-hydroxyacid form by HPLC of the biomass-free filtered broth [5, 10]. The filtered broth containing the β-hydroxyacid form of lovastatin was diluted ten-fold with acetonitrile/water (1:1, v/v) prior to analysis.

Pharmaceutical-grade lovastatin (lactone form) tablets (Nergadan tablets; J. Uriach and Cía., S.A.) were used to prepare the standards for HPLC analyses. Prior to use, the lactone form of lovastatin was converted into its β-hydroxyacid form by dissolving the tablets in a mixture of 0.1 N NaOH and ethanol (1:1, v/v), heating the solution at 50 °C for 20 min, and neutralizing it with HCl. HPLC was done on a Beckman Ultrasphere ODS (250×4.6 mm I.D., 5 μm) column. The column was mounted in a Shimadzu model LC10 liquid chromatograph equipped with a Shimadzu MX-10Av diode array detector. The eluent was a mixture of acetonitrile and 0.1% phosphoric acid (60:40, v/v). The eluent flow rate was 1.5 ml·min^{-1}. The detection wavelength was 238 nm. The sample injection volume was 20 μl.

Results and discussion

Two series of experiments were carried out starting from different batches of spores. Series I experiment comprised two replicates. Series II experiment had four replicates. The lovastatin concentration reproducibility (95% confidence level) in series I and II was ± 16 mg·l^{-1} and ± 4 mg·l^{-1}, respectively.

Time-course production profiles (separate averages for series I and II) for lovastatin are shown in Fig. 1 for cultures spiked with lovastatin at three levels (50, 100, 250 mg·l^{-1}). The control fermentations were not spiked. The *y*-axis in Fig. 1 shows the concentration of lovastatin produced (not including the spiked concentration) normalized to the maximum concentration attained at the end of the corresponding control fermentation, and expressed as a percentage. This way, the two series of experiments could be compared without regard to differences in the final lovastatin concentrations within each series. (The final lovastatin concentrations attained in series I and II experiments were 93 ± 16 mg·l^{-1} and 56 ± 4 mg·l^{-1}, respectively.)

The figure clearly reveals that increasing the concentration of spiked lovastatin from 50 to 100 to 250 mg·l^{-1} progressively reduced the production rate and the final titer of the metabolite. The final titer and productivity declined to 93, 64, and 27%, respectively, of control in the three experiments within series I, and 75, 46, and 18%, respectively, of control in the three experiments of series II. Furthermore, increasing concentration of spiked lovastatin produced a progressive delay of the onset of lovastatin synthesis by the culture (Fig. 1). The added lovastatin did not influence the biomass growth profiles, and all fermentations attained a final biomass concentration of 8.93 ± 0.29 g·l^{-1} in series I and 9.86 ± 0.61 g·l^{-1} in series II.

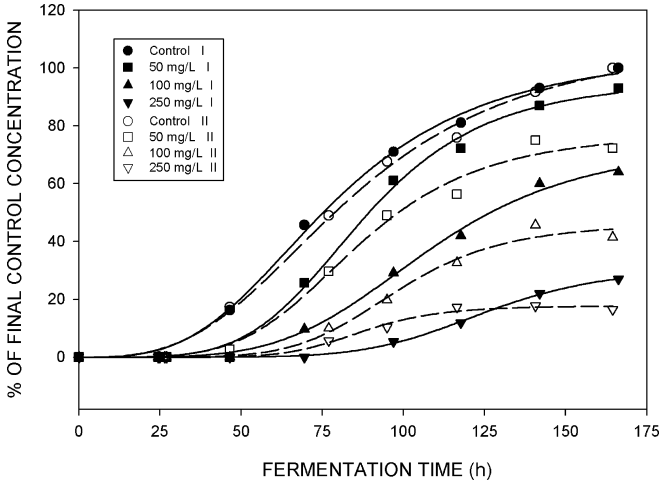

Fig. 1 Lovastatin production profiles as a function of spiked lovastatin concentration

Clearly, a feedback regulatory mechanism exists for lovastatin synthesis in *A. terreus*, and eliminating or suppressing this mechanism can greatly enhance the production of lovastatin. We are currently investigating methods for suppressing the product inhibition of lovastatin fermentation.

Conclusions

Accumulation of lovastatin suppresses its synthesis through a feedback regulatory mechanism in *A. terreus*. Overcoming this effect will greatly enhance the productivity of lovastatin.

Acknowledgements This research was supported by the Ministerio de Ciencia y Tecnología (MYCT) and FEDER (PPQ2000-0032-P4-02).

References

1. Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J, Springer J (1980) Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proc Natl Acad Sci USA 77:3957–3961
2. Casas López JL, Sánchez Pérez JA, Fernández Sevilla JM, Acién Fernández FG, Molina GrimaE, Chisti Y (2003) Production of lovastatin by *Aspergillus terreus*: effects of the C:N ratio and the principal nutrients on growth and metabolite production. Enzyme Microb Technol 33:270–277

3. Endo A, Kuroda M, Tsujita Y (1976) ML-236 A, ML236 B and ML-236 C, new inhibitors of cholesterogenesis produced by *Penicillium citrinum*. J Antibiot 29:1346–1348.
4. Endo A, Kuroda M, Tanazawa K (1976) Competitive inhibition of 3-hydroxy-3-methyl glutaryl coenzyme A reductase by ML-236 A and ML236 B fungal metabolites having hypocholesterolemic activity. FEBS Lett 72:323–326
5. Friedrich J, Zuzek M, Bencina M, Cimerman A, Strancar A, Radez I (1995) High-performance liquid chromatographic analysis of mevinolin as mevinolinic acid in fermentation broths. J Chromatogr A 704:363–367
6. Juzlova P, Martinkova L, Kren V (1996) Secondary metabolites of the fungus *Monascus*: a review. J Ind Microbiol 16:163–170
7. Kumar MS, Jana SK, Senthil V, Shashanka V, Kumar SV, Sadhukhan AK (2000) Repeated fed-batch process for improving lovastatin production. Process Biochem 36:363–368
8. Manzoni M, Rollini M, Bergomi S, Cavazzoni V (1998) Production and purification of statins from *Aspergillus terreus* strains. Biotechnol Tech 12:529–532
9. Manzoni M, Bergomi S, Rollini M, Cavazzoni V (1999) Production of statins by filamentous fungi. Biotechnol Lett 21:253–257
10. Morovján G, Szakács G and Fekete J (1997) Monitoring of selected metabolites and biotransformation products from fermentation broths by high-performance liquid chromatography. J Chromatogr A 763:165–172
11. Novak N, Gerdin S, Berovic M (1997) Increased lovastatin formation by *Aspergillus terreus* using repeated fed-batch process. Biotechnol Lett 19:947–948
12. Sanchez S, Demain AL (2002) Metabolic regulation of fermentation processes. Enzyme Microb Technol 31:895–906
13. Szakács G, Morovján G, Tengerdy RP (1998) Production of lovastatin by a wild strain of *Aspergillus terreus*. Biotechnol Lett 20:411–415