Available online at www.sciencedirect.com
SCIENCE DIRECT®
Enzyme and Microbial Technology 33 (2003) 270–277
ENZYME and MICROBIAL TECHNOLOGY
www.elsevier.com/locate/enzmictec

Production of lovastatin by *Aspergillus terreus*: effects of the C:N ratio and the principal nutrients on growth and metabolite production

J.L. Casas López [a,*], J.A. Sánchez Pérez [a], J.M. Fernández Sevilla [a], F.G. Acién Fernández [a], E. Molina Grima [a], Y. Chisti [b]

[a] *Department of Chemical Engineering, University of Almería, 04120 Almería, Spain*
[b] *Institute of Technology and Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand*

Received 17 July 2002; received in revised form 2 May 2003; accepted 3 May 2003

Abstract

Production of lovastatin and microbial biomass by *Aspergillus terreus* ATCC 20542 were influenced by the type of the carbon source (lactose, glycerol, and fructose) and the nitrogen source (yeast extract, corn steep liquor, and soybean meal) used and the C:N mass ratio in the medium. Use of a slowly metabolized carbon source (lactose) in combination with either soybean meal or yeast extract under *N*-limited conditions gave the highest titers and specific productivity ($\sim$0.1 mg g^{-1} h^{-1}) of lovastatin. The maximum value of the lovastatin yield coefficient on biomass was $\sim$30 mg g^{-1} using the lactose/soybean meal and lactose/yeast extract media. The optimal initial C:N mass ratio for attaining high productivity of lovastatin was $\sim$40. The behavior of the fermentation was not affected by the method of inoculation (fungal spores or hyphae) used, but the use of spores gave a more consistent inoculum in the different runs.

Keywords: Lovastatin; Mevinolin fermentation

1. Introduction

Lovastatin ($C_{24}H_{36}O_5$, Mevinolin, Monacolin K, and Mevacor™) is a potent drug for lowering blood cholesterol. Lovastatin acts by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) [1,2] which catalyzes the rate limiting step of cholesterol biosynthesis. Lovastatin is produced as a secondary metabolite by the fungi *Penicillium* sp. [3], *Monascus ruber* [4,5], and *Aspergillus terreus* [1]. *A. terreus* appears to be the most commonly used producer of this drug [6–11]. Fermentation-derived lovastatin is also a precursor for simvastatin, a powerful semi-synthetic statin commercially available as Zocor™. Simvastatin is obtained via a selective enzymatic deacylation of lovastatin [12].

Lovastatin is generally produced by batch fermentation in complex media. *A. terreus* fermentations are typically carried out at $\sim$28 °C and pHs 5.8–6.3 [10]. The dissolved oxygen level is controlled at $\geq$40% of air saturation [10]. A batch fermentation generally runs for <10 days. Fed-batch fermentations of *A. terreus* have been investigated for producing lovastatin and are said to be superior to batch cultures [6,10]. At least in some cases, pelleted growth of *A. terreus* has yielded higher titers of lovastatin than obtained with filamentous growth [10]. Uncontrolled filamentous growth occurs when using rapidly metabolized substrates. The rapid increase in viscosity accompanied by filamentous growth greatly impedes oxygen transfer and this is said to explain the low titers of lovastatin. Lovastatin productivity of wild strains of *A. terreus* is relatively low, but selected mutants can produce lovastatin titers of $\geq$2200 mg l^{-1} within 12 days in fed-batch fermentations [10].

As with any fermentation product, the culture medium has a significant influence on the yield of lovastatin and its rate of production. Selection and composition optimization of a suitable medium is therefore important for establishing a process for producing lovastatin. Various statistical experimental design strategies are widely used for optimizing fermentation media [13]. Of the major culture nutrients, carbon and nitrogen sources generally play a dominant role in fermentation productivity because these nutrients are directly linked with the formation of the biomass and the metabolite. Also, the nature and concentration of the carbon source can regulate secondary metabolism through phenomena such as catabolic repression. Biosynthesis of lovastatin has been found to depend on the carbon and nitrogen sources, but the results have been inconsistent as the observed effects depend

* Corresponding author.
E-mail address: jlcasas@ual.es (J.L. Casas López).

0141-0229/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0141-0229(03)00130-3

on strain used and culture conditions, that is, the composition and concentration of other culture medium components. [8,9,11].

The present paper reports on the effects of the carbon and nitrogen sources and the C:N mass ratio on *A. terreus* ATCC 20542 fermentation for producing lovastatin. The influence of the C:N ratio was evaluated over the range of 14:1 to 50:1. The lower value of the C:N ratio was selected to correspond closely with the C:N ratio of the *A. terreus* biomass. The amount of carbon was then increased until nitrogen became the growth limiting nutrient. The carbon sources evaluated were glycerol, fructose and lactose. These compounds contained 3, 6 and 12 mol of carbon, respectively, per mole of the compound. The selected carbon sources are considered not to cause catabolic repression as reported for glucose [11]. The nitrogen sources used were yeast extract, corn steep liquor (CSL) and soybean meal. These organic nitrogen sources have been claimed to promote synthesis of lovastatin [8,9,14].

2. Materials and methods

2.1. Microorganism and inoculation

The microfungus used was obtained from the American Type Culture Collection, as *A. terreus* ATCC 20542. The culture was maintained on 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. In separate experiments suspensions of spores and the fungal hyphae were used to inoculate the submerged fermentations. The culture media contained (per liter): 10 g lactose, 8 g yeast extract, 1.51 g KH_2PO_4, 0.52 g $MgSO_4{\cdot}7H_2O$, 0.40 g NaCl, 1 mg $ZnSO_4{\cdot}H_2O$, 2 mg $Fe(NO_3)_3{\cdot}9H_2O$, 0.04 mg biotin, and 1 ml trace element solution. The trace element solution contained (for 1 l of solution): 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 (0.1N) before sterilization.

The 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 (∼2800 × *g*, 5 min) and the solids were resuspended in sterile distilled water. The spores concentration was determined spectrophotometrically at 360 nm.

2.2. Culture conditions

All fermentations were carried out at 28 °C in replicated shake flasks held on a rotary platform shaker (150 rpm). Other details varied with the experimental run, as detailed next.

2.2.1. C:N mass ratio

Cultures for establishing the effects of C:N ratio were conducted in 250 ml shake flasks filled with 100 ml of the medium. Lactose was the carbon source and nitrogen was provided as CSL. The flasks were inoculated with 500 μl of a spore suspension which had been standardized to contain 2×10^8 spores ml^{-1}. The initial spore concentration was high enough not to affect the dimension of the biomass pellets [15]. The culture lasted up to 10 days. The CSL used contained 50% total solids, 6.7% nitrogen and 36.3% carbon, on dry weight per volume basis.

Cultures commenced at a given initial lactose concentration and after 6 days of fermentation a further addition of lactose was made. The culture continued for a total of 10 days. The concentration of initial lactose, the subsequently added lactose and the nitrogen source are indicated in Table 1. The 'equivalent' value of the C:N mass ratio was calculated using the total amount of lactose added (Table 1). In addition to the components in Table 1, the media contained the following (per liter): 1 g KH_2PO_4, 1 g $MgSO_4{\cdot}7H_2O$, 1 g NaCl, and 1 ml trace element solution. The trace element solution contained (for 1 l of solution): 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 (0.1N) before sterilization.

2.2.2. Carbon and nitrogen source screening

Cultures for screening the carbon and nitrogen sources for effects on fermentation performance were conducted in 100 ml shake flasks filled with 50 ml of the medium. The flasks were inoculated with 500 μl of a spore suspension which had been standardized to contain about 2.26×10^8 spores ml^{-1}. The flasks were held on a rotary shaker and harvested on Days 3, 6 and 9 since inoculation. Nine suboptimal media were used, as identified in Table 2.

The media in Table 2 were additionally supplemented with the following components (per liter of medium): 1.51 g KH_2PO_4, 0.52 g $MgSO_4{\cdot}7H_2O$, 0.40 g NaCl, 2 mg $Fe(NO_3)_3{\cdot}9H_2O$, 1 mg $ZnSO_4{\cdot}H_2O$, 0.04 mg biotin and

Table 1
Media compositions used in determining the effects of C:N ratio

Initial lactose concentration ($g\,l^{-1}$)	CSL ($g\,DW\,l^{-1}$)	Initial C:N	Added lactose ($g\,l^{-1}$)	Equivalent C:N
8	5.32	14.4:1	32	50.3:1
16	5.32	23.4:1	24	50.3:1
32	5.32	41.3:1	8	50.3:1

Table 2
Media compositions for screening of C- and N-sources

Experimental run	C-source	C-source concentration ($g\,l^{-1}$)	Medium ($g\,C\,l^{-1}$)	N-source	N-source concentration ($g\,l^{-1}$)	Medium ($g\,N\,l^{-1}$)	C:N ratio
1	Lactose	20.00	8.524	Yeast extract	1.33	0.144	59.19
2	Lactose	20.00	9.663	Soybean meal	3.84	0.326	29.64
3	Lactose	20.00	8.966	CSL	2.66	0.178	50.31
4	Glycerol	20.44	8.524	Yeast extract	1.33	0.144	59.19
5	Glycerol	20.44	9.663	Soybean meal	3.84	0.326	29.64
6	Glycerol	20.44	8.966	CSL	2.66	0.178	50.31
7	Fructose	20.00	8.524	Yeast extract	1.33	0.144	59.19
8	Fructose	20.00	9.663	Soybean meal	3.84	0.326	29.64
9	Fructose	20.00	8.966	CSL	2.66	0.178	50.31

1 ml of the earlier specified trace element solution. The pH was adjusted to 6.5 with NaOH before sterilization.

The C-sources (Table 2) did not contain any nitrogen. The C-content (% by dry weight) of lactose, glycerol and fructose were 39.96, 39.08 and 39.96, respectively. The C- and N-content of CSL are noted in the previous section. The other nitrogen sources had the composition listed in Table 3.

2.3. Analytical methods

2.3.1. Biomass

The biomass dry weight was determined by filtering a known volume of the broth through a 0.45 μm Millipore cellulose filter and freeze drying the solids.

2.3.2. Lovastatin

Lovastatin was determined as its beta hydroxyacid, by high performance liquid chromatography (HPLC) of the biomass-free filtered broth [16]. Because the fungus secretes lovastatin in the beta hydroxyacid form, the assay eliminated the conversion step to the active lactone form of the drug. Using the beta hydroxyacid permitted rapid analysis because this form elutes earlier from a chromatography column than does the lactone form of lovastatin. Also, the beta hydroxyacid is quite stable in solution. The filtered broth containing the beta hydroxyacid form of lovastatin was diluted 10-fold with acetonitrile–water (1:1 by volume) prior to analysis [17].

Pharmaceutical grade lovastatin (lactone form) tablets (Nergadan® tablets; J. Uriach & Cía., S.A.) containing 40 mg lovastatin per tablet were used to prepare the standards for the HPLC analyses. Prior to use, the lactone form of lovastatin was converted to the beta hydroxyacid form by dissolving the tablets in a mixture of 0.1N NaOH and ethanol (1:1 by volume), heating at 50 °C for 20 min, and neutralizing with HCl. The resulting standard stock solution contained 400 mg lovastatin (beta hydroxyacid) per liter. The solution was held at 5 °C until needed.

Table 3
N- and C-content of yeast extract and soybean meal

Nitrogen source	Nitrogen (% of dry wt.)	Carbon (% of dry wt.)
Yeast extract	10.80	39.36
Soybean meal	8.50	43.30

HPLC was performed on a Beckman Ultrasphere ODS (250 mm × 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 by volume). The eluent flow rate was $1.5\,ml\,min^{-1}$. The detection wavelength was 238 nm. The sample injection volume was 20 μl.

2.3.3. Carbon in the culture medium

The total carbon in the biomass-free broth was determined using an elemental analyzer (Leco CHNS-932).

3. Results and discussion

Fermentation profiles of submerged cultures inoculated with suspensions of spores and hyphae are shown in Fig. 1. In view of the comparable results (Fig. 1) of the two methods of inoculation, subsequent fermentations used spore suspensions for inoculation. Because the spore suspensions could be easily quantified for spore concentrations, this method assured a consistent and well-defined inoculum size for the various experiments. The initial spore concentration ($2 \times 10^8\,spores\,ml^{-1}$) used was sufficiently high not to influence the dimension of the biomass pellets (diameter 900–1100 μm) [15].

For the three initial C:N values of 14.4, 23.4 and 41.3, the quantity of nitrogen (i.e. CSL) in the medium was held constant (Table 1) and the initial C-content were varied using lactose. Nitrogen was the limiting nutrient in all cases. As expected, all these cultures attained the same final biomass concentration ($7.0 \pm 0.2\,g\,l^{-1}$) after 10 days of fermentation. The lovastatin production profiles are shown in Fig. 2. Further lactose was added to cultures (see Table 1) on Day 6 (144 h in Fig. 2) so that the final equivalent C:N ratio was 50.3 in all cases. Addition of lactose barely affected the

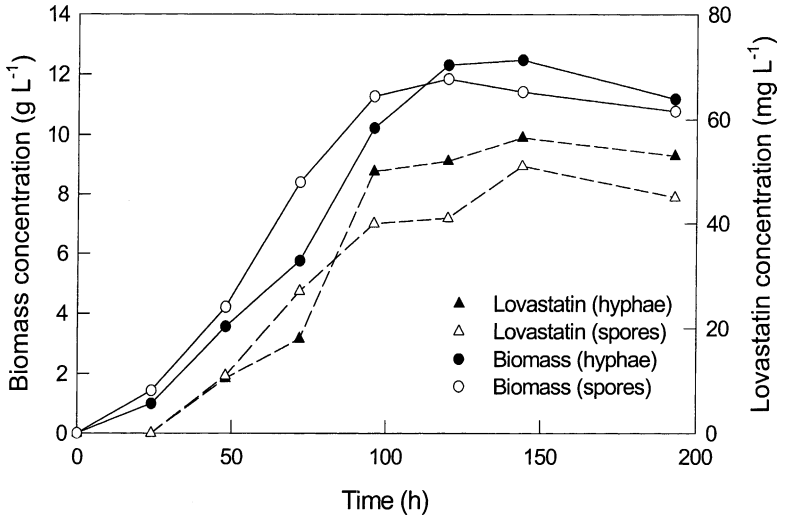

Fig. 1. Comparison of the biomass and lovastatin production profiles in fermentations inoculated with suspensions of spores and hyphae.

total biomass production (final total biomass between 6.8 and 7.2 g l^{-1} in all cases) because of nitrogen limitation; however, the presence of excess carbon under nitrogen limitation greatly enhanced the rate of production of lovastatin (Fig. 2). The final lovastatin concentration was the highest in the culture with an initial C:N value of 41.3 because more carbon was channeled to producing lovastatin ($C_{24}H_{36}O_5$) which contains only carbon, hydrogen and oxygen. The lovastatin production had not ceased at the time of termination of fermentations. Clearly, the biomass produced at a relatively high initial C:N ratio was better adapted to producing lovastatin when supplemented with further carbon.

Production of lovastatin is generally associated with the stationary phase of nitrogen-limited growth when excess carbon can be channeled into secondary metabolism. Lovastatin is synthesized via the polyketide pathway [18,19]. After addition of lactose at 144 h of culture, a lovastatin specific generation rate of 0.242 mg (g biomass)$^{-1}$ h^{-1} was obtained for the three C:N values tested.

In view of these results, lovastatin yield can be improved when carbon is not limiting but growth has been arrested by nitrogen limitation. These results are consistent with the findings of Hajjaj et al. [11]. Relatively low specific production of lovastatin (e.g. 0.034 mg g^{-1} h^{-1}) has been reported [11] in cultures growing at high specific growth rate (e.g. 0.070 h^{-1}) while a higher productivity (e.g. 0.093 mg g^{-1} h^{-1}) is found for lower growth rates (e.g. 0.052 h^{-1}). Apparently, starvation (i.e. no growth because of insufficiency of an essential nutrient) conditions tend to favor the production of lovastatin.

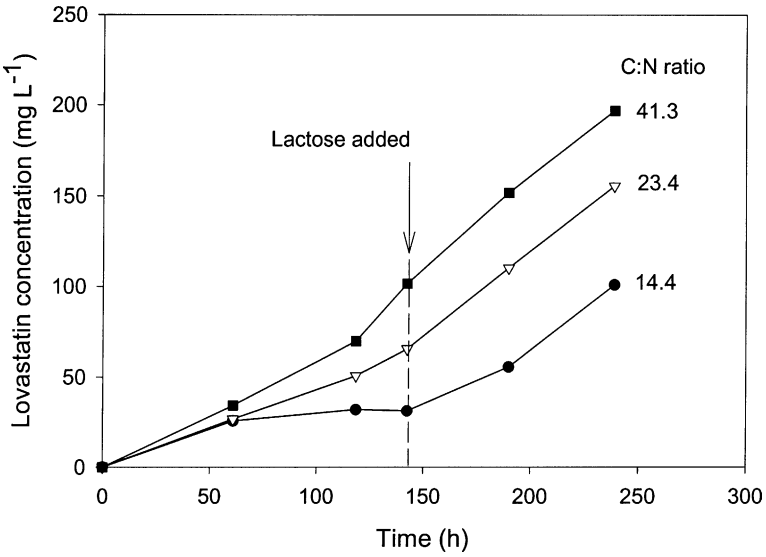

Fig. 2. Lovastatin production for various values of the initial C:N ratio.

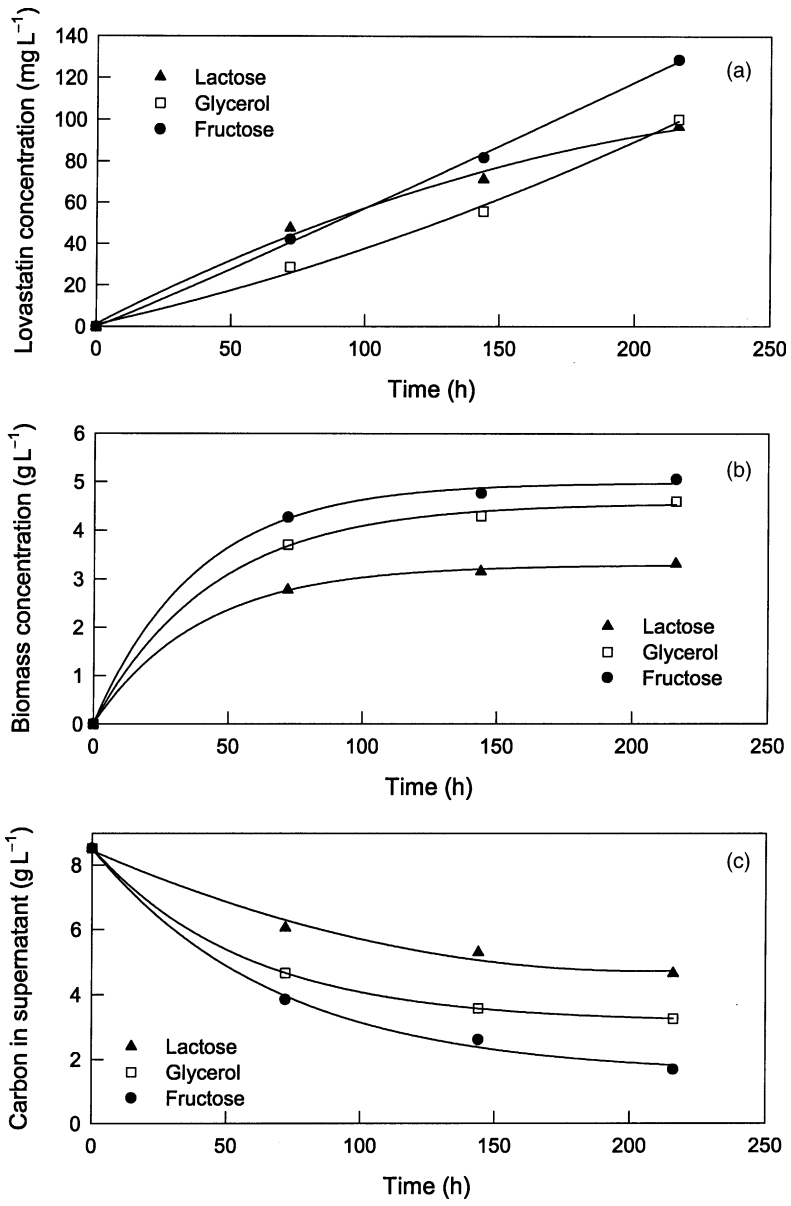

Fig. 3. Lovastatin (a), biomass (b) and residual carbon (c) concentrations profiles for culture on various carbon sources with yeast extract providing the nitrogen.

A second set of experiments examined the effects of various types of nitrogen and carbon sources on the production of lovastatin and biomass. In all cases, nitrogen was provided in growth limiting amounts as yeast extract, CSL or soybean meal. (Nitrogen limitation assured that the biomass concentration remained controlled at $\leq 15\,g\,l^{-1}$, so that agitation and oxygen transfer were not impaired excessively.) The C-sources were lactose, glycerol and fructose. Figs. 3–5 show the time profiles of lovastatin synthesis, biomass production, and residual carbon for cultures carried out with yeast extract, CSL and soybean meal N-sources, respectively. A comparison of the figures (Figs. 3–5) shows that irrespective of the N-source, using fructose as the carbon source gave the highest biomass concentration after 72 h of culture. Growth was much slower when lactose was the C-source. This is reflected in the final biomass yield coefficient ($Y_{X/N}$) values on nitrogen, given in Table 4.

Table 4
Biomass yield on nitrogen ($Y_{X/N}$, g biomass g^{-1} nitrogen) for various combinations of carbon and nitrogen sources

Carbon source	Nitrogen source (biomass yield on nitrogen ($g\,g^{-1}$))		
	Yeast extract	CSL	Soybean meal
Lactose	23.014	20.270	25.135
Fructose	35.090	29.539	24.690
Glycerol	31.917	25.635	25.503

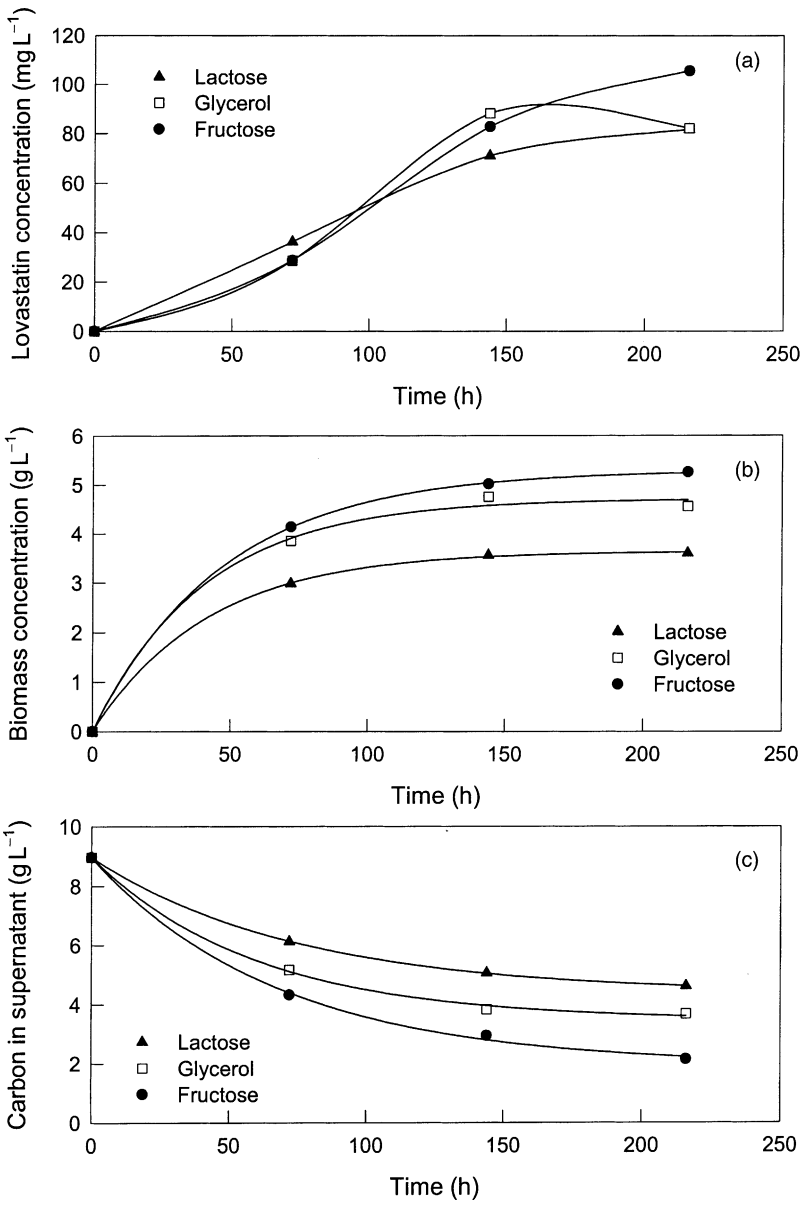

Fig. 4. Lovastatin (a), biomass (b) and residual carbon (c) concentrations profiles for culture on various carbon sources with CSL providing the nitrogen.

The data on residual carbon concentration (Figs. 3–5c) confirmed a faster consumption of fructose than lactose for all the N-sources tested. The final attainable biomass concentration for a given N-source depended on the C-source used (Figs. 3–5b). In comparison with the other N-sources, the relatively high final biomass concentration attained with soybean meal (Fig. 5b) was because of a high initial concentration of nitrogen (326 mg N l^{-1}, Table 2).

Concerning lovastatin, generally, the carbon source that yielded the highest concentration of biomass in N-limited growth also yielded high titers of lovastatin (Figs. 3a and 4a). Using soybean meal as the N-source, the final concentration of the biomass were quite similar for the three C-sources (Fig. 5b). However, the C-sources that produced slowest growth (i.e. lactose) yielded the highest final titer of lovastatin (Fig. 5a). This suggests that the effect of N- and C-sources are interdependent and the specific type of the nutrient source is a relevant consideration. The combinations of lactose and yeast extract (Fig. 3) and lactose and soybean meal (Fig. 5) gave about the same specific productivity of lovastatin, at ∼0.1 mg g^{-1} h^{-1}. The cultures with yeast extract, CSL, and soybean meal began with C:N ratios of 60, 50 and 30, respectively. Thus, in the stationary phase, the soybean meal medium (Fig. 5c) had the least concentration of excess (residual) carbon when compared with the cultures grown on yeast extract (Fig. 3c) and CSL (Fig. 4c).

As shown in Table 4, the biomass yield on nitrogen ($Y_{X/N}$) depended both on the N- and the C-source. Use of yeast extract as the N-source generally gave comparatively high values of $Y_{X/N}$, irrespective of the C-source used.

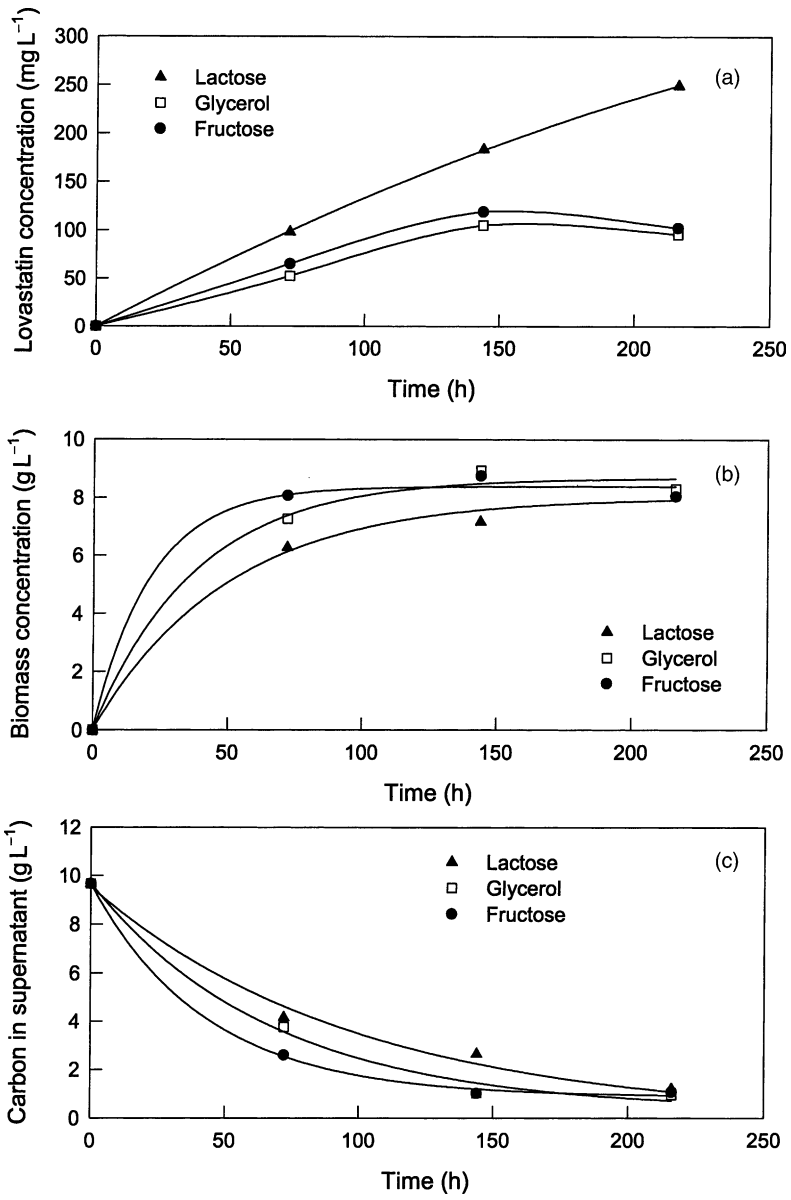

Fig. 5. Lovastatin (a), biomass (b) and residual carbon (c) concentrations profiles for culture on various carbon sources with soybean meal providing the nitrogen.

However, for the combinations of C- and N-sources examined, the range of $Y_{X/N}$ values was relatively narrow (Table 4, $Y_{X/N}$ = 20.3–35.1) compared to the range for the lovastatin yield coefficient ($Y_{L/X}$) on biomass (Table 5, $Y_{L/X}$ = 11.5–30.4 mg g^{-1}). Clearly, selecting an appropriate combination of C- and N-sources was more influential on lovastatin yield than on the biomass yield.

Table 5
Lovastatin yield coefficient on biomass ($Y_{L/X}$, mg lovastatin g^{-1} biomass) for various combinations of carbon and nitrogen sources

Carbon source	Nitrogen source (lovastatin yield on biomass (mg g^{-1}))		
	Yeast extract	CSL	Soybean meal
Lactose	29.196	22.657	30.419
Fructose	25.467	20.079	12.654
Glycerol	21.802	18.032	11.456

4. Concluding remarks

The biomass and lovastatin production performance of *A. terreus* cultures does not depend on whether hyphae or spores are used for inoculation. Media compositions for attaining high biomass and lovastatin production with *A. terreus* are distinctly different. A high productivity and final yield of lovastatin are generally obtained using a slowly metabolized carbon source under conditions of nitrogen limitation. The type of the nitrogen source used affects

the productivity of lovastatin. Yeast extract and soybean meal are the preferred nitrogen sources when compared to CSL. Apparently, the metabolic pathways for the synthesis of lovastatin from carbon are much slower than the pathways that convert carbon to biomass. Therefore, nitrogen limitation (i.e. growth suppression) helps with synthesis of lovastatin by diverting more carbon to its synthesis.

Acknowledgments

This research has been 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, et al. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proc Natl Acad Sci USA 1980;77:3957–61.

[2] Alberts AW. Lovastatin and simvastatin—inhibitors of HMG CoA reductase and cholesterol-biosynthesis. Cardiology 1990;77:14–21.

[3] Endo A, Kuroda M, Tsujita Y. ML-236 A, ML236 B and ML-236 C, new inhibitors of cholesterogenesis produced by *Penicillium citrinum*. J Antibiot 1976;29:1346–8.

[4] Endo A, Kuroda M, Tanazawa K. 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 1976;72:323–6.

[5] Juzlova P, Martinkova L, Kren V. Secondary metabolites of the fungus *Monascus*: a review. J Ind Microbiol 1996;16:163–70.

[6] Novak N, Gerdin S, Berovic M. Increased lovastatin formation by *Aspergillus terreus* using repeated fed-batch process. Biotechnol Lett 1997;19:947–8.

[7] Manzoni M, Rollini M, Bergomi S, Cavazzoni V. Production and purification of statins from *Aspergillus terreus* strains. Biotechnol Tech 1998;12:529–32.

[8] Manzoni M, Bergomi S, Rollini M, Cavazzoni V. Production of statins by filamentous fungi. Biotechnol Lett 1999;21:253–7.

[9] Szakács G, Morovján G, Tengerdy RP. Production of lovastatin by a wild strain of *Aspergillus terreus*. Biotechnol Lett 1998;20:411–5.

[10] Kumar MS, Jana SK, Senthil V, Shashanka V, Kumar SV, Sadhukhan AK. Repeated fed-batch process for improving lovastatin production. Process Biochem 2000;36:363–8.

[11] Hajjaj H, Niederberger P, Duboc P. Lovastatin biosynthesis by *Aspergillus terreus* in a chemically defined medium. Appl Environ Microbiol 2001;67:2596–602.

[12] Daborah RA, Lein J, Conder MJ, Tewalt GL. Enzymatic deacylation of simvastatin. British Patent GB2255974, 1992.

[13] Grothe E, Moo-Young M, Chisti Y. Fermentation optimization for the production of poly(β-hydroxybutyric acid) microbial thermoplastic. Enzyme Microb Technol 1999;25:132–41.

[14] Buckland B, Gbewonyo K, Hallada T, Kaplan L, Masurekar P. Production of lovastatin, an inhibitor of cholesterol accumulation in humans. In: Demain AL, Somkuti GA, Hunter-Cevera JC, Rossmoore HW, editors. Novel microbial products for medicine and agriculture. Amsterdam: Ellesmere; 1989. p. 161–9.

[15] Xu J, Wang L, Ridgway D, Gu T, Moo-Young M. Increased heterologous protein production in *Aspergillus niger* fermentation through extracellular proteases inhibition by pelleted growth. Biotechnol Progress 2000;16:222–7.

[16] Morovján G, Szakács G, Fekete J. Monitoring of selected metabolites and biotransformation products from fermentation broths by high-performance liquid chromatography. J Chromatogr A 1997;763:165–72.

[17] Friedrich J, Zuzek M, Bencina M, Cimerman A, Strancar A, Radez I. High-performance liquid chromatographic analysis of mevinolin as mevinolinic acid in fermentation broths. J Chromatogr A 1995;704:363–7.

[18] Witter DJ, Vederas JC. Putative Diels-Alder-catalyzed cyclization during the biosynthesis of lovastatin. J Org Chem 1996;61:2613–23.

[19] Kennedy J, Auclair K, Kendrew SG, Park C, Vederas JC, Hutchinson CR. Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis. Science 1999;284:1368–72.