HAYATI Journal of Biosciences 22 (2015) 174–180

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Original research article

Optimization of Culture Conditions for Maximal Lovastatin Production by *Aspergillus terreus* (KM017963) under Solid State Fermentation

Praveen Vadakke Kamath, Bhargavi Santebennur Dwarakanath, Avinash Chaudhary, Savitha Janakiraman*

Department of Microbiology and Biotechnology, Bangalore University, JB Campus, Bangalore 560056, India.

ARTICLE INFO

Article history:
Received 25 September 2015
Received in revised form
24 November 2015
Accepted 26 November 2015
Available online 9 February 2016

KEYWORDS:
Aspergillus terreus,
solid state fermentation,
wheat bran,
lovastatin,
optimization

ABSTRACT

Effects of various culture conditions on lovastatin production were investigated in *Aspergillus terreus* (KM017963) grown under solid state fermentation with wheat bran. Lovastatin production was influenced by various physical factors such as pH, temperature, and nutritional factors such as carbon, nitrogen, metal ions/salts etc. Our study established that an initial pH of 6.0, growth temperature of 28 °C −30 °C, inoculum size of 10^8 spores/mL as the optimal physiological culture conditions for maximal production of lovastatin by *A. terreus* (KM017963). The carbon sources, glucose or dextrin when supplemented at 3% (w/w) enhanced lovastatin production by five fold when supplemented as individual component in wheat bran. Addition of metal salts such as $CuSO_4$ (8%), $FeSO_4$ (8%), $CaCl_2$ (10%), NaCl (6%) and $MgSO_4$ (6%) enhanced the production by five fold. Supplementation with nitrogen sources, amino acids, hydrocarbons, surfactants and amino acids did not have any profound effect on lovastatin production.

1. Introduction

Lovastatin ($C_{24}H_{36}O_5$), a fungal secondary metabolite, acts as one of the competitive inhibitors of the enzyme hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase, which catalyses the conversion of HMG-CoA to mevalonate during cholesterol biosynthesis. Inhibition of HMG-CoA reductase, during cholesterol biosynthesis leads to the accumulation of HMG-CoA which is metabolized to simple compound and no lipophilic intermediates are noted (Saleem *et al.* 2013).

Lovastatin is the world's widely prescribed drug to combat hypercholesterolaemia, and was the first statin drug which was approved by United States Food and Drug Administration in the year 1987 (Radha and Lakshmanan 2013). In addition to reducing cholesterol level, lovastatin has been reported to possess anti-cancer property, immuno-modulatory role, anti-inflammatory activity and also plays a role in prevention of neurological disorders, bone disorders etc (Morimoto *et al.* 2006; Seenivasan *et al.* 2008; Barrios and Miranda 2010).

Industrial production of lovastatin by fungi was previously achieved by employing submerged fermentation (SmF). Commercial production of lovastatin is carried out by using *Aspergillus terreus*. In addition to *Aspergillus* sp, *Penicillium* sp, *Monascus* sp, *Paecilomyces* sp, *Trichoderma* sp, *Pleurotus* sp, *Scopulariopsis* sp etc are reported as lovastatin producers (Bizukojc and Ledakowicz 2009; Upendra *et al.* 2013).

SmF is the cultivation of microorganisms in liquid nutrient broth. Selected microorganisms are grown in closed environment containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen. SmF is generally accomplished in batch or continuous culture system. Solid state fermentation (SSF) is defined as the growth of microbes in solid substrates without free flowing aqueous phase. Substrates such as bran, bagasse, vegetable wastes etc are the potential substrates for production of pharmaceutically important bioactive compounds (Renge *et al.* 2012).

Of late, SSF technology is being adapted because of several merits of SSF over SmF. Solid substrates as fermentable sources are tested owing to their low prices, eco-friendly approach, perennial availability, low polluting effluents and easier downstream processing (Praveen and Savitha 2012). In general, several cheaply available agro wastes are reportedly being used as substrates for production of pharmaceutically important metabolites (Osman *et al.* 2011; Jahromi *et al.* 2012). Despite its limitations such as

* Corresponding author.
E-mail address: drsvtj@yahoo.co.in (S. Janakiraman).
Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2015.11.001

chances of contamination, low degree of aeration due to high solid concentration, SSF can lead to significant increase in yield (Mienda *et al.* 2011).

Screening and evaluation of nutritional and environmental requirements of microorganism is an important initial step for bioprocess development for any metabolite under SmF or SSF. Traditional methods of optimization involve supplementation of solid substrates with various C/N/trace elements/surfactants (Pandey *et al.* 2001). The main aim of the present study was to evaluate various environmental and physiological parameters so as to establish optimum growth conditions for the maximum production of lovastatin by the soil isolate *A. terreus* (KM017963) by SSF.

2. Materials and Methods

2.1. Screening of substrates

Numerous substrates [wheat bran, corn kernel, corn cob, corn peel, potato skin, litchi peel, ashgourd seed, orange peel, tamarind shell, groundnut shell, groundnut cake, coconut cake, sooji, rice husk, carrot, saw dust, sugarcane bagasse, green gram bran, gram husk, ragi bran, sesame seeds, *Averrhoa bilimba*, sago, sweet potato, coffee husk, pea peel, mosambi skin, *Psyllium* husk, red rice(whole) and red rice (broken)] were screened for lovastatin production. The spore count was done using haemocytometer. The moisture content was maintained at 70% by adding 1.4 mL of sterile distilled water having $10^{7/8}$ spores/mL, to 2 g of substrate. The flasks were then incubated at 28 °C for 8 days (Jaivel and Marimuthu 2010).

2.2. Extraction

After 8 days of incubation, the solid substrate was dried at 40 °C for 24 hours, gently crushed and extracted with 10 mL of ethyl acetate by shaking at 180 rpm for 2 hours followed by filtration through Whatman No. 1 paper. to One mL of 1% trifluoroacetic acid was added to 1 mL of extract and incubated for 10 minutes (lactonization of hydroxyl acid form of lovastatin). The filtrate was then spotted onto thin layer chromatography (TLC) for identifying lovastatin in crude extract along with an authentic lovastatin reference standard (Reddy *et al.* 2011).

2.3. Thin layer chromatography

The extracted organic phase was concentrated to about 50 µL using a block heater adjusted to 45 °C and applied to a heat activated 20 × 20 mm silica gel TLC plates. Dichloromethane and ethyl acetate (70:30, v/v) were used as mobile phase. All the plates were observed under a hand-held ultraviolet lamp (254 nm) after developing thrice in the same mobile phase and exposed to iodine vapour. For each TLC run, lovastatin authentic standard (Merck, KGaA, Darmstat) was spotted for retention factor (R_f) value comparison and confirmation (Praveen *et al.* 2014).

2.4. High performance liquid chromatography

For high performance liquid chromatography, a C18 column (250 mm × 4.6 mm × 5 mm internal diameter) with diode array detector was used. Acetonitrile and water (acidified with 1.1% phosphoric acid) (70:30 v/v) were used as mobile phase. The eluent flow rate was maintained at 1.5 mL/minute and detection carried out at 238 nm with injection volume of 20 µL (Samiee *et al.* 2003). The production of lovastatin is expressed in mg/g dry weight substrate (DWS). The yield of lovastatin was calculated (Muthumary and Sashirekha 2007). Mevinolin (M2147) (Sigma-Aldrich, Srpuce Street, St. Louis, USA) was used as standard.

2.5. Optimization studies

Unless otherwise stated, wheat bran was used as a sole source of carbon for lovastatin production. Two grams of wheat bran was used with a relative humidity (RH) of 70%. One millilitre of spore suspension ($10^{7/8}$ mL spores) of *A. terreus* (KM017963) was added to the sterilized substrate and incubated at 28 °C for 8 days (Jaivel and Marimuthu 2010).

2.5.1. Effect of carbon sources

Eleven different carbon sources (glucose, lactose, maltose, fructose, sucrose, starch, carboxymethyl cellulose, dextrin, xylose, mannitol and cellulose) were used to assess for their influence on lovastatin production by *A. terreus* KM017963. Each carbon source was tested at different concentrations ranging from 1% to 5% by supplementing them in wheat bran. The inoculated flasks were incubated for 8 days at 30 °C. At the end of the incubation period the yield of lovastatin was determined as mentioned previously.

2.5.2. Effect of nitrogen sources

Seven nitrogen sources were tested for their effect on lovastatin production (ammonium molybdate, ammonium oxalate, ammonium sulphate, ammonium nitrate, yeast extract, malt extract and peptone). The concentrations used were from 1% to 5% by supplementing them in wheat bran. The inoculated flasks were incubated for 8 days at 30 °C and lovastatin yield was determined.

In addition, the following physical and physiological parameters were also tested at different concentrations to determine the optimal concentration for each (Table 1).

2.6. Statistical analysis

All experiments were done in triplicates and statistical analysis were performed by using SPSS version 20. The statistical difference between mean values were accessed by one way analysis of variance through Scheffe post hoc test at significance level ($p < 0.05$).

Table 1. Additional parameters tested for lovastatin production in solid state fermentation.

Parameters	Variable tested	Reference
pH	4.0, 6.0, 7.0 and 8.0	(Chanakya *et al.* 2011)
Temperature	25 °C, 28 °C, 30 °C and 35 °C	(Chanakya *et al.* 2011)
Particle size	0.5 cm, 0.25 cm and fine powder	(Jahromi *et al.* 2012)
Initial moisture	60%, 70% and 80%	(Chanakya *et al.* 2011)
Inoculum size	10^4 $10^7/10^8$ spores	(Chanakya *et al.* 2011)
Hydrocarbons (butyrolactone, dodecane)	0.1%–0.3%	(Bizukojc and Ledakowicz 2009)
Metal ions	10%–40%	(Jia *et al.* 2010)
Surfactants (Tween-20, Tween-80)	10%–40%	(Bizukojc and Ledakowicz 2009)
Amino acids (methionine, histidine and glycine)	10%–40%	(Osman *et al.* 2011)
Sodium acetate	10%–40%	(Osman *et al.* 2011)

Table 2. List of substrates used to screen lovastatin for production in *Aspergillus terreus* (KM017963).

Sl. No.	Substrates	HPLC yield (mg/g DWS)
1	Corn kernel	0.128
2	Corn cob	UD*
3	Corn peel	UD*
4	Potato skin	0.144
5	Litchi peel	UD
6	Ashgourd seed	UD*
7	Orange peel	UD*
8	Tamarind shell	UD*
9	Groundnut shell	UD*
10	Groundnut cake	UD*
11	Coconut cake	UD
12	Sooji	0.200
13	Wheat bran	1.000
14	Rice husk	UD*
15	Carrot	NG
16	Saw dust	UD*
17	Sugarcane bagasse	UD*
18	Green gram bran	UD*
19	Gram husk	UD*
20	Ragi bran	UD*
21	Sesame seeds	UD*
22	*Averrhoa bilimba*	UD*
23	Sago	UD*
24	Sweet potato	UD*
25	Coffee husk	UD*
26	Pea peel	UD*
27	Mosambi skin	UD*
28	Psyllium husk	NG
29	Broken red rice	0.001
30	Red rice(whole)	0.330

DWS = dry weight substrate; HPLC = high performance liquid chromatography; UD and UD*= Undetectable level, NG and NG*= No growth.

3. Results

Various environmental and nutritional parameters are known to exhibit significant impact on yield of secondary metabolites. Additional nutrients to the native solid substrates, sometimes, if not always, influence the production of certain metabolites by acting as an inducer (VanderMolen *et al.* 2013). We identified a soil isolate, *A. terreus* (KM017963) as a potent lovastatin producing strain out of 360 fungi screened (Praveen *et al.* 2014). Amongst the 30 substrates screened, wheat bran showed the highest yield of lovastatin (1.00 mg/g DWS) (Table 2) when compared to other substrates selected for growth and hence chosen for optimization studies.

In this study, we report various environmental and nutritional parameters for the optimum production of lovastatin by SSF using wheat bran as control at 30 °C, 70% RH, coarse particle size which yielded 1.00 mg/g DWS of lovastatin without addition of any supplements (Praveen *et al.* 2014).

3.1. Effect of carbon and nitrogen sources

Our data on the effect of carbon sources as supplement to wheat bran indicated that the simple carbon sugars, glucose and the polysaccharide dextrin influenced lovastatin production in *A. terreus* (KM017963). Optimal concentration of these inducers was investigated by varying the concentration from 1% to 5% and maximum lovastatin production was observed at 3% and at 2% of glucose and dextrin, respectively as compared to control (Figure 1).

The effect of various nitrogen sources, that is, ammonium molybdate, ammonium oxalate, ammonium sulphate, ammonium nitrate, yeast extract, malt extract and peptone (1%, 2%, 3%, 4% and 5% w/v) on lovastatin production was studied. Each component was added at 1%–5% w/v to wheat bran. None of the nitrogen sources showed positive effect on lovastatin production in *A. terreus* (KM017963) (data not shown). These nitrogen sources rather influenced the growth of organisms thereby leading to increase in biomass only.

3.2. Effect of pH and temperature

Maximum lovastatin production (3.60 mg/g DWS) was observed in substrate with pH 6.0 and substantial decrease in production was observed above and below the pH 6.0 (Figure 2).

As far as temperature is concerned, lovastatin production was found to be optimum at 28 °C and 30 °C with a yield of 1.20 and 1.4 mg/g DWS, respectively (Figure 3). Further increase or decrease in temperature lead to poor production of lovastatin.

3.3. Effect of moisture, inoculum size and particle size

Among the several factors that are important for microbial growth and metabolite production under SSF by using a particular substrate, moisture content or water activity, inoculum size and particle size are the most critical factors, which determine the biomass and product formation in fermentation process (Pandey *et al.* 2001). Highest lovastatin yield (3.50 mg/g DWS) in *A. terreus* (KM017693) was observed at 60% moisture content (Figure 4).

In *A. terreus* (KM017963) the optimum inoculum size was found to be 10^8 spores/mL which gave a yield of 3.60 mg/g DWS (Figure 5).

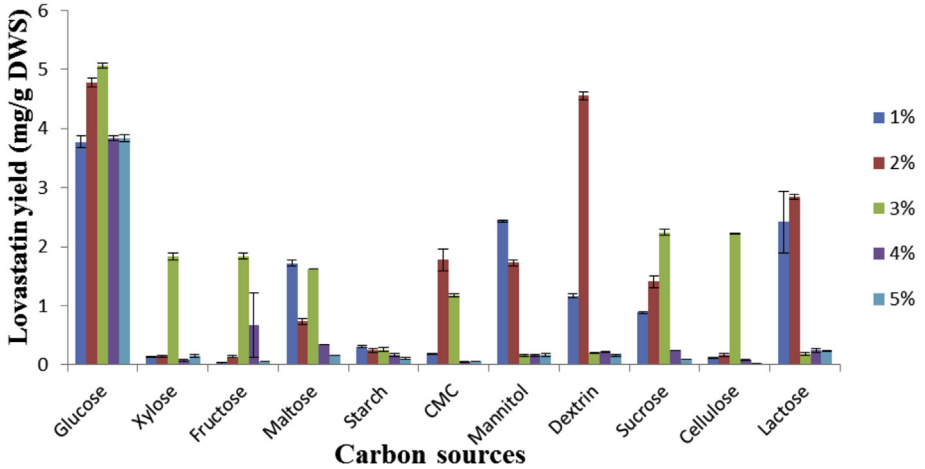

Figure 1. Effect of carbon sources on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. CMC = carboxymethyl cellulose; DWS = dry weight substrate.

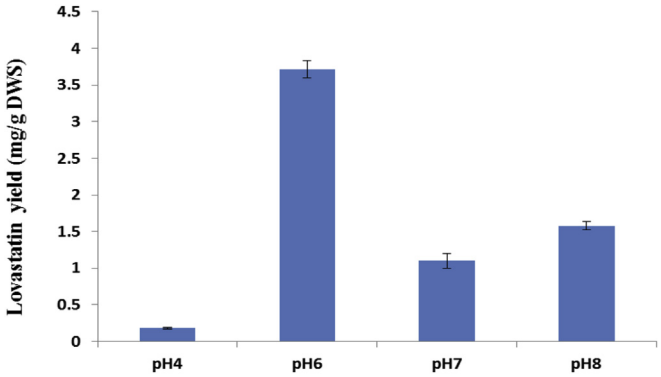

Figure 2. Effect of pH on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

Higher inoculum volume (10^{10} spores/mL) leads to decreased lovastatin production when compared to 10^{8} spores/mL. With low inoculum volume (10^{4} spores/mL), the lovastatin yield (0.1 mg/g DWS) is low because of less biomass resulting in decreased levels of lovastatin.

The particle size also generally influences lovastatin production. Maximum lovastatin production (3.60 mg/g DWS) was observed with medium sized (0.25 mm) wheat bran whereas the fine particle size (0.1 mm) and coarse sized (0.50 mm) (Figure 6) wheat bran did not influence the growth and production of lovastatin in *A. terreus* (KM017963).

3.4. Effect of metal ions, hydrocarbons and surfactants

Our study also confirmed the positive effect of metal ions as individual components on lovastatin production in *A. terreus* (KM017963). Sodium chloride, copper sulphate, calcium chloride, ferrous sulphate and magnesium sulphate as individual components at 6%, 8% and 10% enhanced the lovastatin yield by minimum of five fold (5.0–6.5 mg/g DWS) (Figure 7). However combination of metal salts tested did not favour lovastatin production.

Our study did not show any marked increase in lovastatin yield with any of the hydrocarbon or surfactant incorporated (Figure 8).

3.5. Effect of amino acid and sodium acetate

Sodium acetate showed no positive effect on lovastatin production in *A. terreus* (KM017963) (Figure 9) and addition of amino acids such as methionine, histidine and glycine did not show any positive effect on lovastatin production (Figure 10).

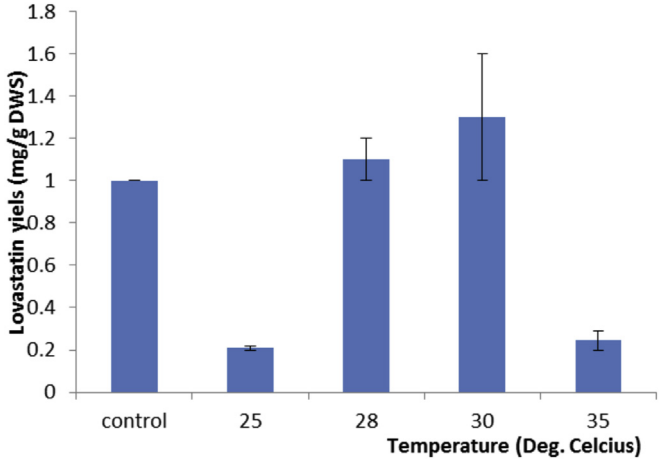

Figure 3. Effect of temperature on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

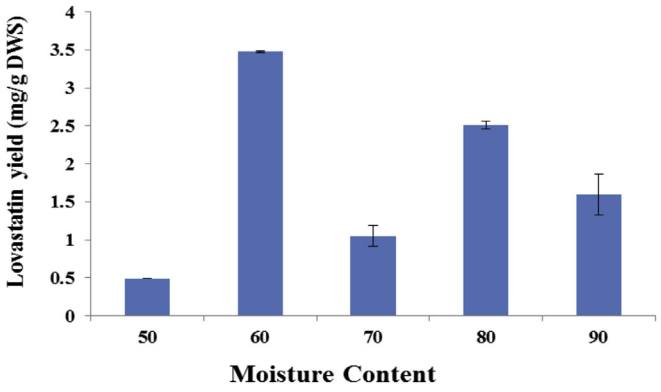

Figure 4. Effect of moisture content on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

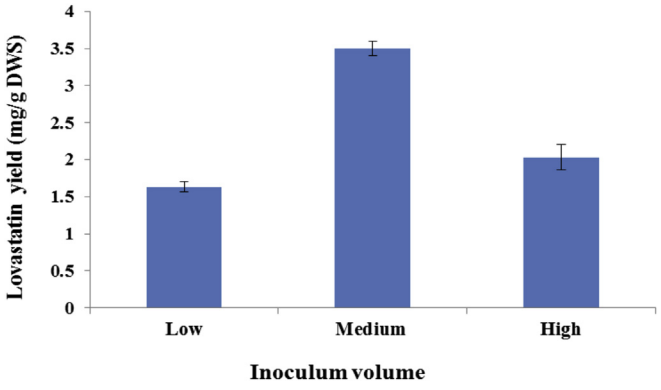

Figure 5. Effect of initial inoculum size on lovastatin production in *A. terreus* (L1). Low: 10^{4} spores/mL; medium: 10^{8} spores/mL; high: 10^{10} spores/mL. Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

4. Discussion

In recent years, researchers have focused much on SSF for commercial production of industrially important metabolites. Wheat bran is generally considered as a complete solid substrate

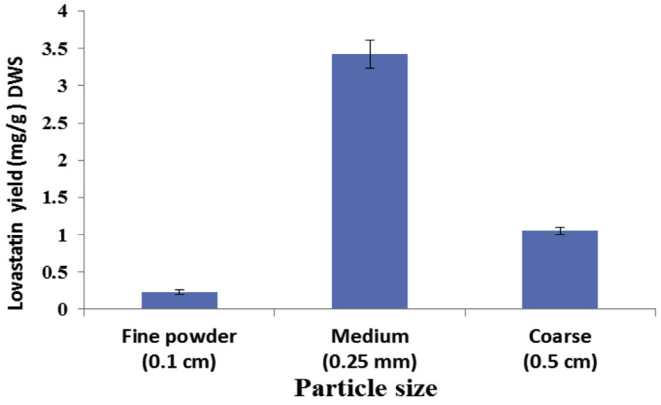

Figure 6. Effect of particle size on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

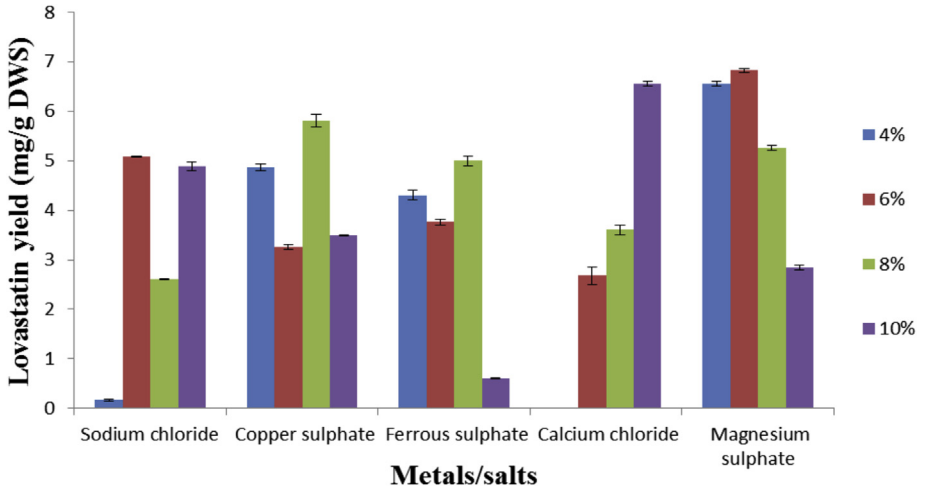

Figure 7. Effect metal ions on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

for growth and metabolite production of microbes consisting of protein, fats and polysaccharides such as arabinoxylans, cellulose and lignin, but, however, lacks readily soluble sugars (Stevenson *et al.* 2012). The aim of the present work was to study the influence of few growth supplements such as readily soluble sugars, nitrogen, metal salts, surfactants etc.

In the present study, of 11 carbon sources tested, glucose and dextrin at 3% and 2% respectively, gave the highest yield of lovastatin. It is obvious that glucose being a readily soluble monosaccharide gets metabolized by the fungal isolates rather quickly although interestingly, dextrin, a low molecular weight carbohydrate derived from the hydrolysis of starch seems to induce the production of lovastatin. Lactose did not increase lovastatin production in our study although it is reported previously that addition of lactose to solid substrate has positive effect on lovastatin production (Chanakya *et al.* 2011). On the contrary, none of the disaccharides used in our study had any positive effect on lovastatin production. Generally, nitrogen sources (organic and inorganic) have a vital role in increasing lovastatin production. Yeast extract and sodium nitrate are shown to have significant impact on increasing lovastatin production (Xu *et al.* 2005; Chanakya *et al.* 2011). However, none of the nitrogen sources (organic and inorganic) tested with *A. terreus* (KM017963) showed any effect on increasing lovastatin production.

Physical factors such as temperature, pH, moisture content, inoculum volume and particle size were also investigated for increased lovastatin production. Variations in pH affect transport of molecules across cell membrane and suppression of fungal growth. A pH of 6.0 was found to be suitable for lovastatin production in *A. terreus* (KM017963) as compared to reports of pH 5.0 being suitable in *A. fischeri* and *A. flavipes* (Valera *et al.* 2005; Chanakya *et al.* 2011).

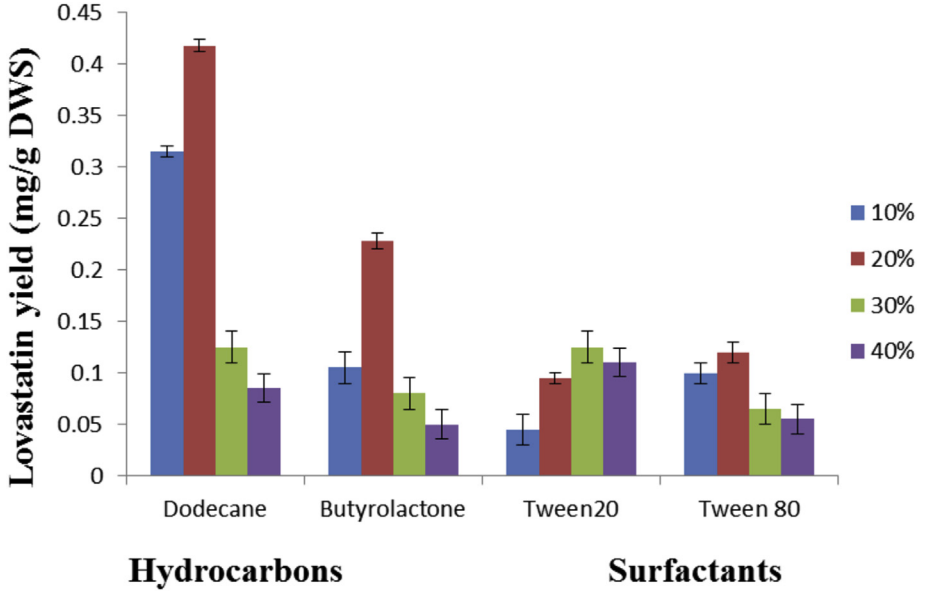

Figure 8. Effect of hydrocarbons and surfactants on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

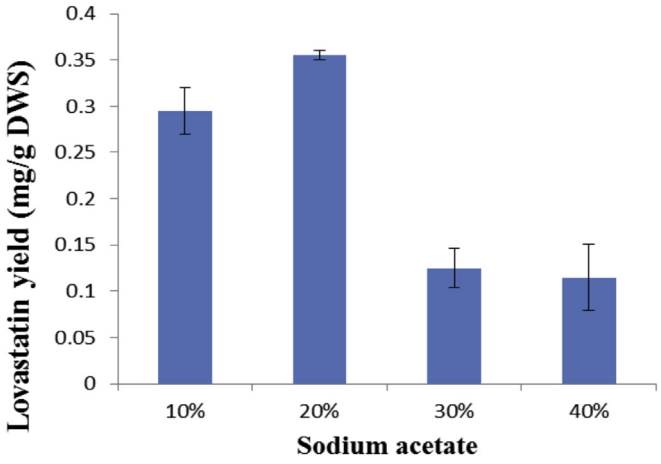

Figure 9. Effect of sodium acetate on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

In mesophilic organisms the temperature between 26 °C and 30 °C significantly influences metabolite production, enzyme activity, protein function and cell viability (Chanakya *et al.* 2011). Our investigations on effect of temperature on lovastatin production are in accordance to temperature of 30 °C being most suitable for lovastatin production as reported in *A. fischeri* and *A. flavipes* and *M. ruber* (Valera *et al.* 2005; Xu *et al.* 2005; Chanakya *et al.* 2011).

Yield of lovastatin was observed to be optimum at 60% which is reported to be suitable for lovastatin production (Xu *et al.* 2005; Chanakya *et al.* 2011). As the moisture content increased, the yield of lovastatin decreased correspondingly, presumably, because of the aggregation of substrate particles, decrease of void volume and thereby reducing the aeration, growth and metabolite production (Jahromi *et al.* 2012). Under low moisture conditions, the available oxygen is sufficient, but the water content is not enough to support upright metabolic activity and heat dissipation (Valera *et al.* 2005; Aparna and Reddy 2012). Inoculum volume governs lovastatin titre. Higher volume causes rapid nutrient depletion before the completion of growth period, thus resulting in poor yield of the product (Chanakya *et al.* 2011). Initial inoculum volume of 10^7 spores/mL gave the highest yield of lovastatin in *A. terreus* (KM017963) which is the most widely reported spore volume (Jaivel and Marimuthu 2010).

Surface area of the particle in solid substrate plays a major role for initial attachment, accessibility of nutrients and subsequent colonization by microbes. In our study, a particle size of 0.25 mm was found to be optimum for lovastatin production. A particle size of 0.4 mm was reported to be favourable for lovastatin production on *A. flavipes* (Valera *et al.* 2005) whereas in *A. terreus* (KM017963) 0.5 mm particle size did not favour lovastatin production.

In lovastatin biosynthetic pathway, the enzyme diketide synthase catalyses the attachment of 2 methyl butyric acid chain to monacolin J and to form monacolin L. This enzyme is regulated by the gene *lov*F. The function of *lov*F is enhanced in the presence of divalent metal ions such as Zn or Fe in the medium (Jia *et al.* 2010). Accordingly in our study, the metal ions such as sodium chloride, copper sulphate, calcium chloride, ferrous sulphate and magnesium sulphate increased lovastatin yield by minimum of five fold individually, but when supplemented in combination had moderate inhibitory action on lovastatin production.

Surfactants play a vital role in altering cell membrane permeability and to ease the passage of secondary metabolites in to the external environment. Also, addition of hydrocarbons in general when added as supplements under submerged growth condition aids in oxygen supply, increased mycelial growth and product formation. A significant increase in lovastatin yield by three fold (940 mg/L) in SmF with supplementation of butyrolactone was recorded (Bizukojc and Ledakowicz 2009). However, none of the surfactants and hydrocarbons had any positive impact on lovastatin production under SSF in our strain of *A. terreus* (KM017963).

Lovastatin biosynthetic pathway gets initiated with acetate units being linked to each other in head-to-tail fashion to form two polyketide chains. Later, the amino acid methionine donates its methyl group to the growing chain and forms the functional lovastatin (Osman *et al.* 2011). However, addition of sodium acetate and amino acids did not have any effect on production of lovastatin in *A. terreus* (KM017963).This implies that sodium acetate and amino

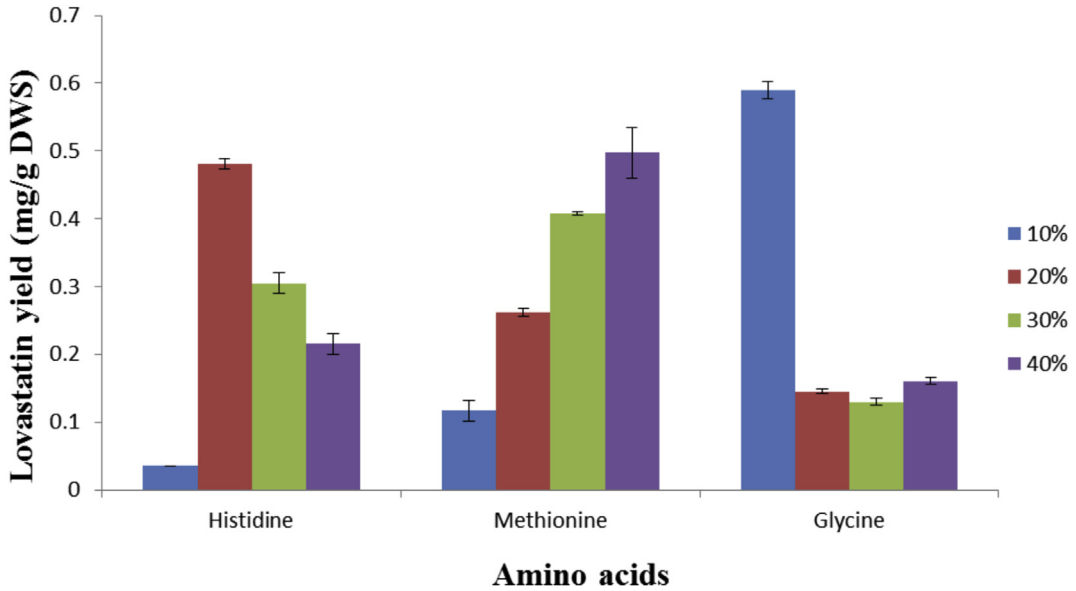

Figure 10. Effect of amino acids on lovastatin production in *A. terreus* (L1). Control: 2 g of wheat bran with relative humidity of 70%, pH 7.0, $10^{7/8}$ spores/mL, 28 °C, 0.5 cm particle size. DWS = dry weight substrate.

acids required for the biosynthetic pathway of cellular functions are sufficiently produced by organisms when grown in SSF.

Very few reports are available on optimization of lovastatin production under SSF and this study is one of the few. Some of the essential nutrients available in solid substrate can be of sub-optimal concentrations or absent. Basic supplementation of required nutrients could act as an inducer for growth and/or production of any metabolite. Therefore, an attempt was made in this study to optimize the solid substrate with varied supplements which resulted in few significant findings which could be exploited for large scale production of lovastatin in *A. terreus* under SSF.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank the financial support provided by Science and Engineering Research Board, Department of Science and Technology, Govt. of India vide grant number DST/SO-F.No.SERB.SR/SO/PS/046/2011.

References

Aparna GD, Reddy DSR. Production of Lovastatin by *Aspergillus parasiticus* NCIM 696 using rice bran under solid state fermentation. 2012, J. Chem. Bio. Phy. Sci. 2(1); 284–91.

Barrios GJ, Miranda RU. 2010. Biotechnological production and applications of statins. *Appl Microbiol Biotech* 85:869–83.

Bizukojc M, Ledakowicz S. 2009. Physiological, morphological and kinetic aspects of lovastatin biosynthesis by *Aspergillus terreus*. *Biotech J* 4:1–61.

Chanakya P, Latha PM, Srikanth M. 2011. Solid state fermentation for the production of lovastatin production by *Aspergillus fischeri*. *Res J Pharm Sci Biotech* 1(1):9–13.

Jahromi MH, Liang JB, Ho WH, Mohamad R, Goh YM, Shokryazdan P. 2012. Lovastatin production by *Aspergillus terreus* using agro-biomass as substrate in solid state fermentation. *J Biomedicine and Biotech*. http://dx.doi.org/10.1155/2012/196264.

Jaivel N, Marimuthu P. 2010. Optimization of lovastatin production in solid state fermentation by *Aspergillus terreus*. *Intl J Engg Sci Technol* 2(7):2730–3.

Jia Z, Zhang X, Zhao Y, Cao X. 2010. Enhancement of lovastatin production by supplementing polyketide antibiotics to the submerged culture of *Aspergillus terreus*. *Appl Biochem Biotech* 160:2014–25.

Mienda SB, Idi A, Umar A. 2011. Microbiological features of solid state fermentation and applications—an overview. *Res Biotech* 2(6):21–6.

Morimoto K, Janssen WJ, Fessler MB, McPhillips KA, Borges VM, Bowler RP, Xiao Y, Kench JA, Hensom PM, Vandivier RW. 2006. Lovastatin enhances clearance of apoptotic cells (efferocytosis) with implications for chronic obstructive pulmonary disease. *J Immuol* 176:7657–65.

Muthumary J, Sashirekha S. 2007. Detection of taxol, an anticancer drug, from selected Coelomycetous fungi. *Indian J Sci Tech* 1:1–10.

Osman ME, Khattab OH, Zaghlol GM, El-Hameed RM. 2011. Screening for the production of cholesterol lowering drugs (lovastatin) by some fungi. *Aus J Basic Appl Sci* 5:698–703.

Pandey A, Soccol CR, Rodrigoez-Leon JA, Nigam P. 2001. Solid State Fermentation in Biotechnology—Fundamentals and Applications, first ed. Asiatech Publications Inc.

Praveen VK, Savitha J. 2012. Solid state fermentation: an effective method for lovastatin production by fungi over submerged fermentation. *E3 Journal of Biotech Pharma Res* 3(2):15–21.

Praveen VK, Bhargavi SD, Savitha J. 2014. Endophytic fungi: a poor candidate for the production of lovastatin. *British Microbiol Res J* 4(12):1511–20.

Radha KV, Lakshmanan D. 2013. A review: lovastatin production and applications. *Asian J Pharma Clincal Res* 6(3):21–6.

Reddy DSR, Latha DP, Latha KPJH. 2011. Production of lovastatin by solid state fermentation by *Penicillium funiculosum* NCIM 1174. *Drug Invention Today* 3(6):75–7.

Renge C, Khedkar SV, Nandurkar NR. 2012. Enzyme synthesis by fermentation method: a review. *Sci Rev Chem Comm* 2(4):585–90.

Saleem F, Ambreen A, Saleem Y, Naz1 S, Ahmad A, Syed Q. 2013. Production and optimization of lovastatin by solid state fermentation using *Aspergillus terreus*. *Global J Sci Res* 1(2):33–41.

Samiee SM, Moazami N, Haghighi S, Mohseni S, Mirdamadi A, Bakhtiari MR. 2003. Screening of lovastatin production by filamentous fungi. *Iran Biomed J* 7(1): 29–33.

Seenivasan A, Shubahgar S, Arvindan R, Viruthagiri T. 2008. Microbial production and biomedical applications of lovastatin. *Ind J of Pharma Sci* 70:701–9.

Stevenson L, Phillips F, Sullivan K, Walton J. 2012. Wheat bran: its composition and benefits to health, a European perspective. *Int'l J Food Sci Nut* 63(8):1001–13.

Upendra RS, Khandelwal Pratima, Amiri ZR, Shwetha L, Ausim M. 2013. Screening and molecular characterization of natural fungal isolates producing lovastatin. *Microbial Biochem Tech* 5(2):025–30.

Valera HR, Gomes J, Lakshmi S, Gururaja R, Suryanarayan S, Kumar D. 2005. Lovastatin production by solid state fermentation using *Aspergillus flavipes*. *Enz Microb Tech* 37:521–6.

VanderMolen KM, Raja HA, El-Elimat T, Oberlies NH. 2013. Evaluation of culture media for the production of secondary metabolites in a natural products screening program. *AMB Express* 3(1):71–7.

Xu B, Wang Q, Jia X, Sung C. 2005. Enhanced lovastatin production by solid state fermentation of *Monascus ruber*. *Biotechnol Biochem Engg* 10:78–84.