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Production of Mevastatin by Solid-State Fermentation Using Wheat Bran as Substrate

[1]M. ZafferAhamad, [1]Bibhu Prasad Panda, [2]Saleem Javed and [1]Mohd. Ali
[1]Pharmaceutical Biotechnology Laboratory, Faculty of Pharmacy,
[2]Molecular Biology and Biotechnology Laboratory, Faculty of Science,
Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, India-110062

Abstract: A Modified solid-state fermentation was used to produce mevastatin by *Penicillium citrinum* NCIM 768 using wheat bran as carrier in glycerol and urea based medium as moistening agents. Initially maize, rice, wheat, barley, wheat bran were compared as carrier. Wheat bran was the most suitable carrier, as it did not show agglomeration during fermentation process resulting in better heat and mass transfer during fermentation and higher product yields. The best combination of physiochemical parameters during fermentation process was found to be 28°C incubation temperature, 4.5 medium pH, 20 g of wheat bran of particle size 0.701-1.0 mm at a inoculum volume 2 mL, after 144 h of incubation in a humidity chamber of 70% relative humidity resulted in mevastatin yield of 0.0554 mg mL^{-1} in fermentation broth.

Key words: Mevastatin, solid-state fermentation, wheat bran

Introduction

Mevastatin a hypocholesterimic agent has been an attractive molecule over the last few decades. Mevastatin and its hydroxyl derivative, Mevastatin is an inhibitor of 3-hydroxyl-3-methyl glutaryl coenzyme A (HMG CoA) reductase. The rate-limiting enzyme in cholesterol synthesis in humans and useful drugs against artherosclerosis (Endo, 1985). Pravastatin can be obtained by the biotransformation of mevastatin by *Streptomyces carbophilus* (Manzoni and Rollini, 2002).

Mevastatin produced by submerged fungal fermentation of glucose medium using *Penicillium citrinum* NCIM 768 (Chakravarti and Sahai, 2002) and in the recent year. There has been increasing interest in the use of Solid-State Fermentation (SSF) (Doelle *et al*., 1992; Lonsane *et al*., 1985; Couto and Sanroman, 2006). This is because SSF has a lower energy requirement, higher product yield with little risk of bacterial contamination, generate less wastewater and environmental concerns regarding disposal of solid waste (Lu *et al*., 1997; Rhaghavarao *et al*., 2003).

Wheat bran, wheat, maize, rice, barley are produced abundantly in India and these can be utilized as a carrier for SSF and the present research was undertaken to find suitable carrier to optimize process conditions including particle size, incubation temperature, inoculation volume, pH of production medium, incubation time, substrate content for the production of mevastatin by SSF.

Materials and Methods

Microorganism

Penicillium citrinum NCIM 768 was obtained from NCL, Pune, India. Was maintained by Potato Dextrose Agar slants stored at 4°C and sub cultured every 4 weeks (Chakravarti and Sahai, 2002).

Corresponding Author: Bibhu Prasad Panda, Pharmaceutical Biotechnology Laboratory, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, India-110062
Tel: +91-11-26059688/5627 +91-11-5563138, 6663138 Fax: +91-11-26059663

Chemical

All chemicals were analytical grade from Merck, India.

Materials

High-grade wheat, maize, barley, wheat bran were obtained from local market of New Delhi, India. And were studied in the bioreactor (Growtek, Tarson India, Calcutta, India) for production of mevastatin.

Inoculum Preparation

Spore solution (10^7 mL^{-1}) of *Penicillium citrinum* NCIM 768 was prepared using glycerol water medium (3% glycerol in distilled water) Inoculum was prepared in seed medium. The composition of seed medium was glucose 20 g L^{-1}, glycerol 30 g L^{-1}, Peptone 8 g L^{-1}, $NaNO_3$ 2 g L^{-1}, $MgSO_4$ 1 g L^{-1} were dissolved in water soluble extract of soybean meal (40 g soybean in cheese cloth was kept in 1000 mL of distilled water at 4°C under stirring conditions for 6 days) pH of the medium was kept at 6.3, sterilized at 121°C for 20 min and 15 Psig (Chakravarti and Sahai, 2002). The seed medium was inoculated with spore suspension at 1% v/v incubated at 28°C and 220 rpm in an orbital shaker for 26 h (Chakravarti and Sahai, 2002).

Experimental Set up (Fermentation)

Solid substrate were placed in flot of growtek bioreactor (Kar *et al*., 1999), were inoculated with *Penicillium citrinum* NCIM 768 and liquid medium containing Glycerol 3.5 g L^{-1}, urea 0.54 g L^{-1}, $NaNO_3$ 2.5 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, KH_2PO_4 1 g L^{-1} and NaCl 0.5 g L^{-1} pH 6.3 (Chakravarti and Sahai, 2002). It was then sterilized at 121°C for 20 min at 15 Psig and after it had cooled; it was inoculated with seed culture of *Penicillium citrinum* NCIM 768 and incubated in a humidity chamber for mevastatin production under different conditions. After fermentation, culture broth was centrifuged to separate mycelial biomass and supernatant containing mevastain was processed for mevastain estimation.

Estimation of Mevastatin

Fermentation broth was adjusted to pH 6.5 with either diluted acid (aq. H_3PO_4) or alkali (aq. NaOH). The broth was diluted 5 times with absolute ethyl alcohol, filtered with whattman filter paper No. 1 and absorbance was read at 238 nm using UV Spectrophotometer (Chakravarti and Sahai, 2002).

Results

Comparison and Selection of the Carrier

Initially maize, rice, wheat, barley and wheat bran were used as carrier for production of mevastatin by SSF. At 70% relative humidity; 28°C incubation temperature. The mevastatin production concentration on wheat bran medium was 0.0394 mg mL^{-1} after 6 days of fermentation (Table 1).

Table 1: Effect of different carriers on mevastatin production

Carriers	Mevastatin conc. (mg mL^{-1})
Maize	0.0335
Wheat bran	0.0394
Wheat	0.0296
Rice	0.0267
Barley	0.0240

Effect of Particle Size of The Carrier

The wheat bran of five different particle size was used to get the optimal size for the maximum production of mevastatin viz., M1 (particles size between 2.0 and 4.0 mm), M2 (particles size between 1.4 and 2.0 mm), M3 (particles size between 1.0 and 1.4 mm), M4 (particles size between 0.710 and 1.0 mm) and M5 (particles size between 0.2 and 0.3 mm). Fermentation was carried out at 70% relative humidity, 28°C incubation temperature .The maximum concentration of mevastain in medium M1, M2, M3, M4 and M5 was 0.480, 0.0423, 0.0440, 0.0482 and 0.0401 mg mL^{-1} after 6 days of fermentation. The maximum mevastatin productivity was also maximum in M1 media .The lowest mevastatin production was in M5 media (Table 2).

Effect of Temperature of Incubation

The fermentation process at 70% relative humidity was carried out at 5 different temperature of incubation to get optimal incubation temperature for maximum production of mevastatin viz T1 (24°C), T2 (26°C), T3 (28°C), T4 (30°C) and T5 (32°C) with wheat bran of particle size between 0.710 and 1.00 mm (Table 3). It was found that at 28°C optimum concentration of mevastatin 0.0492 mg mL^{-1} was obtained after 6 days of incubation.

Effect of Seed Volume (Inoculum Volume)

Fermentation was carried out with different inoculum from 0.5 to 2.5 mL to get optimum inoculum volume for maximum production of mevastatin with wheat bran of particle size between 0.710 and 1.00 mm, temperature of incubation at 28°C and at relative humidity of 70%. It was found that at inoculum volume of 2 mL gave maximum mevastatin concentration of 0.0493 mg mL^{-1} after 6 days of incubation (Table 4).

Effect of pH of The Production Medium

Keeping other conditions at optimum level (i.e., temperature of incubation at 28°C, relative humidity at 70%, particle size of wheat bran between 0.710-1.00 mm). It was found that maximum mevastatin production was 0.0552 mg mL^{-1} when production medium was at initial pH of 4.5 (Table 5).

Table 2: Effect of particle size of the carrier on mevastatin production

Particle size (mm)	Mevastatin conc. (mg mL^{-1})
2.0-4.0	0.0480
1.4-2.0	0.0423
1.0-1.4	0.0440
0.0701-1.0	0.0482
0.2-0.3	0.0401

Table 3: Effect of temperature of incubation on mevastatin production

Temperature (°C)	Mevastatin conc. (mg mL^{-1})
24	0.0435
26	0.0435
28	0.0492
30	0.0425
32	0.0407

Table 4: Effect of seed volume on mevastatin production

Inoculum volume (mL)	Mevastatin conc. (mg mL^{-1})
0.5	0.0376
1.0	0.0389
1.5	0.0388
2.0	0.0493
2.5	0.0398

Table 5: Effect of pH of the production medium on mevastatin production

pH	Mevastatin conc. (mg mL^{-1})
3.5	0.0390
4.0	0.0385
4.5	0.0552
5.0	0.0480
5.5	0.0483

Table 6: Effect of substrate weight on mevastatin production

Weight of substrate (g)	Mevastatin conc. (mg mL^{-1})
5	0.0251
10	0.0318
15	0.0395
20	0.0554
25	0.0412
30	0.0360
35	0.0296
40	0.0210

Effect of Substrate Weight

The amount of substrate was varied from 5-40 g while keeping the production medium constant (50 mL) while keeping other parameters at optimum level. It was found that maximum mevastatin concentration of 0.0554 mg mL^{-1} was found when substrate weight was kept at 20 g (Table 6).

Discussion

In the present study solid state fermentation was carried out by tacking different solid substrate such as maize, rice, wheat, barley and wheat bran, their efficiency was tested for mevastastin production. Solid substrate used other than wheat bran causes agglomeration, which in turn affect mixing resulting in heat accumulation, improper heat transfer and reducing the mevastatin production. Significant improvements in mevastatin production were realized with wheat bran at particle size of 0.701-1.0 mm. The reason for this could be that small particle size provide higher porosity, resulting in better heat and mass transfer and greater surface area facilitating higher substrate availability to the fungus results higher production of mevastatin (Kumar *et al*., 2003), at inoculum volume of 0.5-2.5 mL. There is reduced fungal biomass concentration results increased utilization of substrate for fungal secondary metabolites production i.e., mevastatin. Acidic environment at pH 4.5 favours transfer of metal ion into fungal cells required for metabolic reactions of the organism resulting in better product formation (Kar *et al*., 1999; Lekha and Lonsane, 1997). Mevastatin production was optimum at 144 h. of incubation due to initial increasing enzyme activity up to 14 h and subsequent decrease may be due to catabolic reaction (Chatterjee *et al*., 1996). The increase in substrate weight beyond 20 g i.e., increase in solid substrate bed height in the float of growtek bioreactor resulted in high heat accumulation and improper heat transfer in solid state bioreactor therefore reducing the product formation (Kar *et al*., 1999).

In the conclusion, economically mevastatin can be produced by solid-state fermentation using wheat bran, which are produced abundantly in India. The best combination of physiochemical parameters at optimal level of 28°C, 4.5 pH, 20 g of wheat bran of particle size 0.701-1.0 mm at a inoculum volume 2 mL, after 144 h of incubation in a humidity chamber of 70% relative humidity resulted in mevastatin yield of 0.0554 mg mL^{-1} in fermentation broth. Physiochemical parameters that were optimized by one-factor-at-a-time in the present study can be further optimized by different statistical methods for higher production of mevastatin.

References

Chakravarti, R. and V. Sahai, 2002. Optimization of compactin production in chemically defined production by *Penicillium citrinum* using statistical method. Process. Biochem., 38: 481-486.

Chatterjee, R., A. Dutta, R. Banerjee and B.C. Bhattacharyya, 1996. Production of tannase by solid-state fermentation. Bioprocess. Eng., 14: 159-162.

Couto, S.R. and M.A. Sanroman, 2006. Application of solid-state fermentation to food industry. A review. J. Food. Eng., 76: 291-302.

Doelle, W.H., D.A. Mitchel and C.E. Roltz, 1992. Solid Substrate Cultivation. Elsevier Applied Science, London, New York, pp: 7-16.

Endo, A., 1985. Compactin (ML 236 B) and related compound as potential cholesterol-lowering agent that inhibit HMG Co-A reductase. J. Med. Chem., 28: 1401-1425.

Kar, B., R. Banerjee and B.C. Bhattacharyya, 1999. Microbial production of gallic acid by modified solid-state fermentation. J. Ind. Microb. Biotech., 23: 173-177.

Kumar, D., V.K. Jain, G. Shanker and A. Srivastava, 2003. Citric acid production by solid-state fermentation using sugarcane bagasse. Process. Biochem., 38: 1731-1738.

Lekha, P.K. and B.K. Lonsane, 1997. Production and application of tannin acyl hydrolase: State of art. Adv. Applied Microbiol., 44: 215-260.

Lonsane, B.K., N.P. Gildayl and S.V. Ramakrishna, 1985. Engineering aspects of solid-state fermentation. Enzyme. Microb. Tech., 7: 258-265.

Lu, M., J.D. Brook and I.S. Madox, 1997. Citric acid production by solid-state fermentation in packed bed reactor using *Aspergillus niger*. Enzyme. Microb. Tech., 21: 392-397.

Manzoni, M. and M. Rollini, 2002. Biosynthesis and biotechnological production of stains by filamentous fungi and application of these cholesterol lowering drugs. Applied Microbiol. Biot., 58: 555-564.

Rhaghavarao, K.S.M.S., T.V. Ranganathan and N.G. Karanth, 2003. Some engineering aspects of solid state fermentation. Biochem. Eng. J., 13: 127-135.