



GLYCEROL IMPROVES THE PRODUCTIVITY OF THE LIPASE INHIBITORY ACTIVITY OF *STREPTOMYCES COELICOLOR*

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ABSTRACT

Lipase inhibitory activity was confirmed in the submerged cultures of *Streptomyces coelicolor* during time course studies and addition of glycerol increased the production of this activity. Lipase inhibitory activity was increased with the increasing concentration of glycerol up to 2 %. There was a dramatic increase of the inhibitory activity on the 7th day of the submerged batch fermentation. However, there was no significant increase in the biomass suggesting the secondary metabolite characteristic of the inhibitory activity. The presence of glycerol in the submerged cultures increased the inhibitory activity by 3.4 times compared to the control on the 7th day. During fed-batch fermentation, feeding of glycerol to the submerged culture led to the increase of inhibitory activity by 9.7 times. These results suggest that glycerol can activate the pathways responsible for the production of the inhibitory activity. Improving the productivity of this lipase inhibitor will benefit the biotech and pharma sectors since this inhibitor can be a candidate for treating obesity due to its ability to inhibit lipase.

KEY WORDS: Submerged culture, *Streptomyces coelicolor*, glycerol, lipase inhibitor, obesity



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INTRODUCTION

With the improved economic status of the people the prevalence of obesity is increasing at an alarming rate. Obesity is primarily related to the lipid metabolism and the enzymes involved with this metabolism can be selectively targeted for developing anti obesity drugs. Lipases catalyze the hydrolysis of triglycerides to glycerol & fatty acids and lipases play an important role in the lipid metabolism. Recently, obesity treatments are focussing on the inhibition of absorption of dietary triglycerides by inhibiting the pancreatic lipase.^{1,2} Atherosclerosis can also be treated by using lipase inhibitors. Lipstatin, a pancreatic lipase inhibitor isolated from *Streptomyces toxytricini* and it inhibits irreversibly.^{3,4} However, it does not inhibit other pancreatic enzymes, phospholipase A2 and trypsin even at higher concentrations. Tetrahydrolipstatin also popularly known as orlistat is a lipase inhibitor obtained by hydrogenation of lipstatin. Orlistat inhibits all three gastro-intestinal lipases (gastric lipase, pancreatic lipase and cholesterol ester hydrolase) in vitro.^{5,6} Orlistat is the only commercially available lipase inhibitor for obese patients. Panclitics (A-E) are another type of lipase inhibitors isolated from *Streptomyces* NR 0619.^{7,8} Structurally, panclitics A-E are analogues of tetrahydrolipstatin which contains a beta-lactone and a N-formyl leucine ester. Panclitics and tetrahydrolipstatin will inhibit pancreatic lipase irreversibly. However, panclitics are not as potent as tetrahydrolipstatin. Ebelactones (A & B) of *S. aburaviensis* strongly inhibit the pancreatic lipase.⁹ Intestinal fat absorption is inhibited by Ebelactone B in rats, in a dose-dependent manner. Valilactone isolated from the shake culture of the *S. albolongus*, potently inhibited the hog pancreatic lipase.¹⁰ Esterastin that isolated from the fermentation broth of *S. lavendulae* competitively inhibited the hog pancreas lipase.¹¹ Cyclopostins are another group of lipase inhibitors that inhibit hormone-sensitive lipase and they are isolated from *Streptomyces* DSM 13381.¹² Another lipase inhibitor, (E)-4- Aminostyryl acetate was reported from the cultures of *S. vayuensis*.¹³ A natural preservative ϵ -poly- L-lysine isolated from *S. albulus* was shown to inhibit both human and porcine pancreatic lipases.^{14,15} Another lipase inhibitor, shamistatin was isolated from *Streptomyces* which is a cyclic di-ketone (Shamsiya *et al.*, 2015).¹⁶ Plant essential oils were shown to inhibit the lipase activity of the *Malassezia globosa* which is a fungus.¹⁷ Recently, we have identified a lipase inhibitory activity from the submerged cultures of *S. coelicolor* and there is no reported lipase inhibitor from this species.¹⁸ This activity was confirmed and its productivity was improved in the present study.

MATERIALS AND METHODS

Microbial culture and reagents

Culture of *S. coelicolor* was obtained from microbial type culture collection, Chandigarh, India and maintained on the agar slants containing the medium, yeast extract (0.4 %), malt extract (1 %) and glucose (0.4 %). Orlistat, porcine pancreatic lipase and *p*-Nitrophenyl

butyrate (PNPB) were obtained from Sigma. Ethyl acetate was obtained from Spectrochem, India.

Submerged fermentation

Culture was grown in the 500 ml Erlenmeyer flasks containing 200 ml liquid medium (pH 7.0) comprised of glucose (5%), yeast extract (0.5%) and NaCl (0.3%) for different time periods at 28°C and 150 rpm.^{19,20} Additionally either glycerol or respective oil (1%) was added to the medium, while studying their effects on the productivity of the inhibitory activity by *S. coelicolor*. Different concentrations (0-2.4 %) of glycerol were added to the above medium during the optimization studies. While carrying out fed-batch fermentation and other studies 2 % glycerol was used.

Preparation of extracts

After growing the culture for different periods at submerged fermentation conditions, total culture was dried at 40°C and dry biomass was calculated in grams. One gram of dry biomass was extracted with 10 ml of ethyl acetate at room temperature and 150 rpm for 3h. Then the mixture was centrifuged at 10,000 rpm for 30 min and supernatant was transferred in to another container and dried at 40°C. Dried extract was dissolved in to the 1 ml of DMSO, diluted 1 in 50 with water and 0.4 ml of it was used for the assaying lipase inhibitory activity.²

Lipase inhibitory assay

Pancreatic lipase (20 units) was pre-incubated with the diluted extract (0.4 ml) for 60 min at 37°C before the addition of the substrate. The residual activity (%) of the lipase was determined as described below. The lipase substrate, PNPB (200 μ M) was added to the above mixture and the reaction was carried out at 37°C for 30 min and the released *p*-nitrophenol was measured at 410 nm. The lipase activity was determined as the μ moles of *p*-nitrophenol released per min. A positive control (orlistat) was included in the assay and the percentage of inhibited enzyme activity was calculated by referring to the control having diluted solvent instead of the extract.¹⁸

RESULTS AND DISCUSSION

Species of *Streptomyces* produce various metabolites that have biomedical applications. Few species like *S. toxytricini*, *S. aburaviensis*, *S. albolongus* were also reported to produce lipase inhibitors.¹ Recently, we have identified the lipase inhibitory activity from the submerged cultures of *S. coelicolor*.¹⁸ This species was known to produce antibiotics like actinorhodin, methylenomycin, undecylprodigiosin and perimycin.²¹⁻²⁴ However, lipase inhibitor was not reported from *S. coelicolor*. In the submerged cultures of *S. coelicolor*, there was a notable lipase inhibitory activity on the 5th day of fermentation and there was 2.3 times increase in the lipase inhibitory activity from 6th to 7th day and there was no significant change afterwards (Table 1). There was no significant increase in the biomass from 6th day onwards (Table 1), but there is a drastic increase in the inhibitory activity on the 7th day which suggests that the compound could be a secondary metabolite.

Table 1

Time course study on the production of lipase inhibitory activity in the submerged culture of *S. coelicolor*.

Fermentation time (day)	Dry Biomass (g/L)	Lipase inhibitory activity (%)
0	0.40± 0.11	1.0±0.43
1	0.63±0.16	1.21±0.30
2	1.18±0.30	2.21±0.26
3	2.21 ±0.33	2.59±0.61
4	3.15 ±0.36	4.21±0.62
5	4.21 ±0.37	8.23±1.12
6	5.32 ±0.35	12.18±1.47
7	5.58 ±0.42	28.6±3.18
8	5.43 ±0.31	31.9±2.57
9	5.49 ±0.47	30.66±1.97
10	5.12 ±0.36	29.83± 3.15

n = 2

Glycerol was utilized by different microorganisms like *Clostridium butyricum*, *Yarrowia lipolytica* and *Cupriavidus necator* to produce industrially important metabolites like 1,3-propanediol, citric acid and polyhydroxyalkanoates respectively.²⁵⁻²⁷ Addition of glycerol to the fermenting medium with *S. coelicolor* drastically increased the lipase inhibitory activity compared to the control (Figure 1). However, the addition of olive, coconut or ground nut oil decreased the lipase inhibitory activity (Figure 1). This suggests the inhibition of metabolic pathways involved in the synthesis of lipase

inhibitor by the oils. Hence, oils cannot be used as a raw source for the production of lipase inhibitor by *S. coelicolor*. These results were in agreement with earlier study on the production of streptolipin, a lipase inhibitor from *S. vayuensis*.²⁸ The culture showed the increasing lipase inhibitory activity with the increasing concentration of glycerol and reached highest at 2 % glycerol (Table 2). However, there was no significant change in the biomass at different concentrations of glycerol (Table 2) suggesting the secondary metabolic nature of the lipase inhibitor.

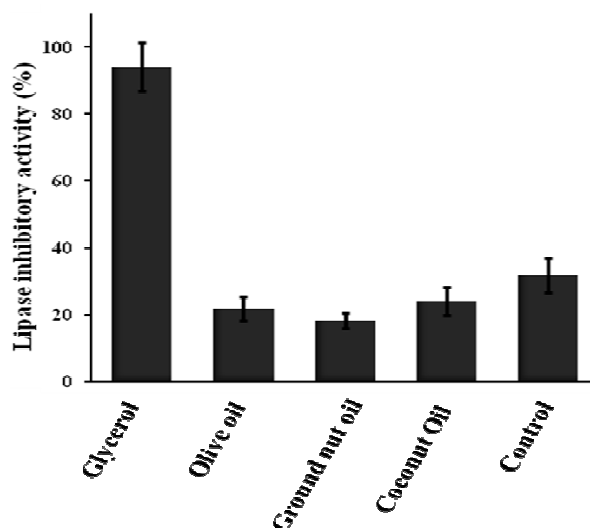


Figure 1

Effect of glycerol and oils on the production of lipase inhibitory activity by *S. coelicolor*.

Table 2

Productivity of lipase inhibitory activity in the presence of different concentrations of glycerol on the 7th day of fermentation by *S. coelicolor*.

Glycerol concentration (%)	Dry Biomass (g/L)	Lipase inhibitory activity (%)
0	5.17±0.7	24.49±3.3
0.4	5.22 ±0.17	30.53±3.93
0.8	5.34±0.76	41.43±5.80
1.2	5.56 ±1.23	51.76 ±2.9
1.6	5.64 ±1.10	63.26±6.5
2	5.81±0.98	96.83±5.13
2.4	5.77 ±0.95	92.56±4.66

n = 7

Incorporation of 2 % glycerol in to the submerged cultures at the beginning of the fermentation led to the production of notable lipase inhibitory activity on the 4th day of fermentation and the inhibitory activity was

increased on the sub-subsequent days (Table 3). There was a drastic increase in the lipase inhibitory activity on the 6th day itself (3.8 times of that on 5th day) and reached maximum on the 7th day and inhibitory activity

was declined afterwards (Table 3). In the presence of glycerol, the activities of the lipase inhibitor were much higher compared to the culture grown without glycerol (compare Tables 1 and 3). On the 7th day, activity of the lipase inhibitor was 3.4 times higher in the presence of glycerol compared to the control. However, there was no significant change in the biomass after 6th day in both the cases (Tables 1 and 3). Statistical analysis showed the P values of 0.00416 and 0.102759 for lipase inhibitory activity and dry biomass respectively after comparing the results with that obtained in the absence of glycerol (Table 1). P value of 0.00416 indicate very significant statistical difference for lipase inhibitory activity and P value of 0.102759 for dry biomass indicate non-significant statistical difference for dry biomass. These results suggest that lipase inhibitor levels

increased very significantly in the presence of glycerol, even though biomass didn't change much. Fed-batch fermentation was specifically used to study the effect of a compound or nutrient on the biomass and targeted metabolite. Hence, in another experiment, control culture was fed with 2 % glycerol after the sufficient biomass built up (6th day) and after 1 day of feeding the inhibitory activity shot up (Figure 2) by 9.7 times, whereas, in the control it was only 2.3 times increase. However, in either case there was no significant change in the biomass and these results indicate that glycerol might activate the pathways that lead to the production of the lipase inhibitory activity. Hence, fed-batch fermentation can be used for the economic production of the lipase inhibitor from *S. coelicolor*.

Table 3
Time course studies on the production of lipase inhibitory activity in the *S. coelicolor* culture containing 2 % glycerol.

Fermentation time (day)	Dry Biomass (g/L)	Lipase inhibitory activity (%)
0	0.48±0.17	1.23±0.56
1	0.55±0.12	1.61±0.53
2	1.15±0.25	1.69±0.53
3	2.07±0.23	6.18±1.5
4	3.01±0.43	12.36±1.44
5	4.09±0.49	23.76 ± 2.6
6	5.29±0.48	92.15±5.06
7	5.81±0.50	97.27±2.5
8	5.99±0.15	90.31±2.02
9	5.76±0.64	89.61±2.01
10	5.88±0.49	89.65±4.01

P = 0.00416 (***) with respect to lipase inhibitory activity after comparing with that in Table 1
P = 0.102759 (ns) with respect to dry biomass after comparing with that in Table 1
n = 2

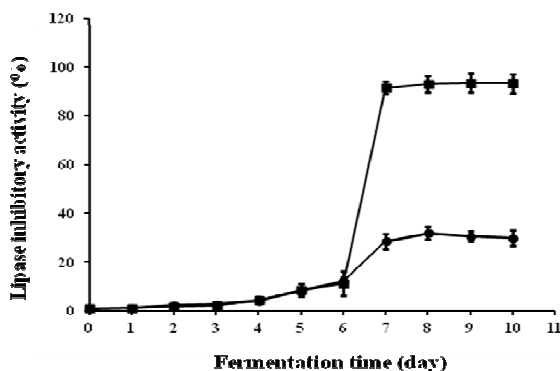


Figure 2
Time course studies on the production of lipase inhibitory activity in the batch and fed-batch fermentations of *S. coelicolor*.

CONCLUSION

Production of lipase inhibitory activity in the submerged cultures of *S. coelicolor* was confirmed. Addition of glycerol increased its productivity in a dose dependent manner. On 7th day, there was a sudden increase in the inhibitory activity, whereas there was no much change in the biomass suggesting the nature of the compound as secondary metabolite. The inhibitory activity was 3.4 times higher in the presence of glycerol compared to the control on the 7th day. In the fed-batch experiments, addition of glycerol to the culture after the sufficient biomass built-up led to the 9.7 times increase in the inhibitory activity suggesting the activation of metabolic pathways that lead to the production of inhibitory activity

by the glycerol. Characterization of this inhibitory activity will give the insights on the nature of the molecule and chemical modifications required to improve its potency. Purification and characterization studies are under way.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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