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CHARACTERIZATION AND KINETICS OF LIPASE INHIBITORY ACTIVITY OF STREPTOMYCES TENDAE

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ABSTRACT

The Streptomyces tendae was grown in the submerged cultures and the lipase inhibitory activity was assyed by using porcine pancreatic lipase. The inhibitory activity was extracted with ethyl acetate and purified by using HPLC. The inhibitory activity was characterized by UV-Vis spectrophotometry and LC-MS and its kinetic studies were done with Lineweaver-Burk plots. Lipase inhibitory activity was identified in the submerged cultures of *S. tendae* and its productivity was higher on the 7th day of fermentation. This inhibitory activity was eluted into the diethyl ether fraction while purifying on silica gel chromatogram and purified compound showed a single peak on HPLC. The compound showed the λ_{max} of 254 nm, IC_{50} of 147.58 µg/ml and mass of 254 amu. Mass suggested it as a small molecule. Kinetic studies suggested that this molecule inhibits the porcine pancreatic lipase in a competitive manner.

KEYWORDS: *Streptomyces tendae , Pancreatic lipase, HPLC, LC-MS and Lipase inhibitor*

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INTRODUCTION

Obesity cases are increasing nowadays due to the changed life style of the people and obesity is due to the problems associated with the functioning of enzymes associated with the lipid metabolism which can be targeted for developing anti-obesity drugs. Lipases hydrolyse the triacylglycerols into glycerol & fatty acids and lipases play an important role in the metabolism of lipids. One of the recent approaches for the treatment of obesity is to target the absorption of triglycerides by inhibiting the pancreatic lipase .[1,2] Inhibitors of lipase will also helpful during atherosclerosis treatment. Esterastin and valilactone produced in the cultures of *Streptomyces lavendulae* and *S. albolongus* respectively inhibited the hog pancreas lipase.[3,4] Well known pancreatic lipase inhibitor, lipstatin is isolated from *Streptomyces toxytricini* [5, 6] and tetrahydrolipstatin (Orlistat, Ro 18-0647), a more potent lipase inhibitor was obtained by hydrogenation of lipstatin [7, 8] and it is the only commercially available lipase inhibitor available for treating obesity. Analogues of orlistat were isolated from *Streptomyces sp.* NR 0619 and they are named as panclicins A, B, C, D and E.[9,10] But, they are not a potent inhibitors compared to orlistat. Ebelactones A and B, another type of pancreatic lipase inhibitors were isolated from *S. aburaviensis*[11] and cyclipostins, hormone-sensitive lipase inhibitors were isolated from *Streptomyces sp*. DSM 13381.[12] Yet another lipase inhibitor, (E)-4- Aminostyryl acetate was isolated from *S. vayuensis*.[13] Epsilon-poly-L-lysine was recently isolated from *S. albulus* and shown to inhibit porcine and human pancreatic lipases. [14] Recently, essential oils from the plant sources were shown to inhibit the extracellular lipase activity of the fungus, *Malassezia globosa.*[15] More recently, shamistatin, a lipase inhibitor was reported from the soil *Streptomyces* species.[16] We have isolated a pancreatic lipase inhibitory activity from the cultures of *S. coelicolor* in the present study and no lipase inhibitor was reported from this species.

MATERIALS AND METHODS

Organism and reagents

Culture of *S. tendae* was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained on the agar slants having 0.4 % glucose, 0.4 % yeast extract, 0.4 % meat extract and 0.2 % $CaCO_3$ (pH 7.2). Porcine pancreatic lipase, *p*-Nitrophenyl butyrate (PNPB) and orlistat were obtained from Sigma. Ethyl acetate and other solvents were obtained from Spectrochem, India.

Lipase inhibitory assay

Porcine pancreatic lipase (20 units) was pre-incubated with the extract (0.4 ml) obtained from the bacterial culture for 60 min at 37°C. Then, the substrate, PNPB (200 µM) was added and the residual lipase activity (%) was determined by carrying out the reaction at 37°C for 30 min. The released *p*-nitrophenol was quantified spectrophotometrically at 410 nm and the activity of lipase was determined as the µ moles of *p*-nitrophenol released per min. Orlistat was used as positive control and the % of enzyme inhibited was calculated by referring to the control that has solvent instead of the bacterial extract.

Purification of lipase inhibitory activity

Around 5 L culture of the *S. tendae* grown in the medium containing glucose (0.5 %), glycerol (2 %), soya bean meal (2 %), yeast extract (0.5 %) and NaCl (0.3 %) was extracted with equal volume of ethyl acetate and centrifuged at 10,000 rpm for 20 min. Organic layer was concentrated by rotary evaporator and the concentrate was loaded on the silica gel column and eluted with different solvents in the order of increasing polarity. The major inhibitory activity was obtained in diethyl ether and further purified by HPLC (column C18- 250 x 4.6 mm, 30^0C) by using the solvent system, triethyl amine buffer: acetonitrile (60:40) at the flow rate of 1ml/min.

Characterization of lipase inhibitory activity

One ml of purified compound (200 µg/ml) dissolved in DMSO was scanned from 190 to 1100 nm by using UV-Vis Spectrophotometer in order to find the λ_{max}. The porcine pancreatic lipase was incubated with different concentrations of purified compound (0-240 µg/ ml) and then lipase assay was carried out and % of lipase activity was obtained at each concentration of the compound. Then IC_{50} was obtained by plotting % of lipase activity against concentration of purified compound. Mass of the compound was identified by LC-MS. The compound (50 µl) was injected and separated on the column, Inersil ODS-3 (250x4.6) mm, 5µ with the flow rate of 1ml/min by using the solvent system, acetonitrile (0.05%): glacial acetic acid (30:70). The compound was subjected to MS/MS fractionation and scanned in positive ion mode to obtain the mass of fragments.

Kinetic studies

Lipase assay was carried out by using different concentrations of the substrate, PNPB (0-300 µM) and porcine pancreatic lipase (20 units). In another set of experiments purified inhibitory molecule (150 µg) was added to the lipase before the addition of different concentrations of the substrate to the mixture. In both the cases lipase activities were calculated at the different concentrations of the substrate and the reciprocals of the substrate concentrations were plotted against the reciprocals of the respective lipase activities to obtain Lineweaver-Burk plots. From these plots, K_m and V_{max} values were calculated to find the kind of inhibition exhibited by the purified compound.

RESULTS AND DISCUSSION

The significant level of porcine pancreatic lipase inhibitory activity was observed in the submerged culture *S.tendae* on the 4th day of ferementation (Fig 1) and levels increase with time and reaches maximum on the 7th day of fermentation and decrease afterwards (Fig 1). However, the lipase inhibitory activity was not reported earlier from the culture of *S. tendae* and this inhibitory activity was isolated and characterized in this study.

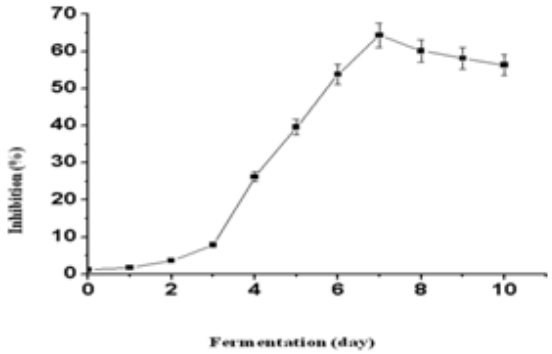

Figure1
Graph showing the production of lipase inhibitory activity in the submerged culture of S.tendae at different fermentation times

The lipase inhibitory activity was purified by using the entire culture that was fermented for 7 days and the details were described in the 'materials and methods' section. There was a single peak at 259 nm (Fig 2) after the analysis of the purified compound by HPLC using PDA detector suggesting the purity of the sample.

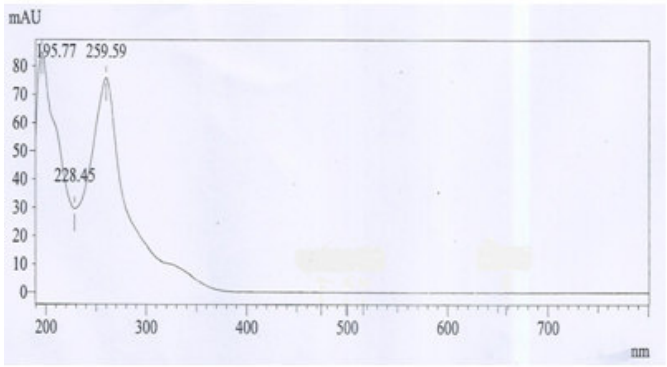

Figure 2
Chromatogram of the purified lipase inhibitory activity after HPLC

The compound showed the λ_{max} of 254 nm after scanning the purified compound from 190 to 1100 nm by using UV-Vis Spectrophotometer and which is close to the peak obtained in the HPLC chromatogram. The compound showed the IC_{50} value of 147.58 µg/ml on the porcine pancreatic lipase (Fig 3). This is higher than the IC_{50} value (0.65 µg/ml) of orlistat on the same lipase and substrate.[17] Nevertheless, chemical modification of this compound might make it a good candidate for further studies.

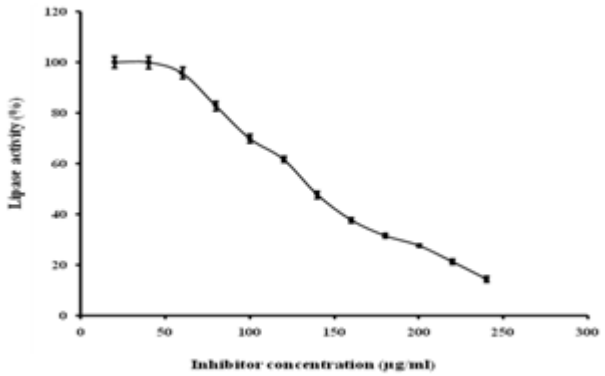

Figure 3
Graph showing the inhibition of porcine pancreatic lipase at the different concentrations of the purified compound (IC_{50} =147.58 µg/ml)

The molecular mass of the compound was determined to be 254 since mass spectrum showed M+1 as 255 (Fig 4). The purified inhibitory molecule was used to study its inhibitory pattern on the porcine pancreatic lipase. The V_{max} value didn't change after the addition of the molecule (Fig 5), whereas, the K_m value increased in the presence of the inhibitory molecule (Fig 5). This pattern is typical to the competitive inhibitor and these kinetic studies suggested that the purified compound is a competitive lipase inhibitor.

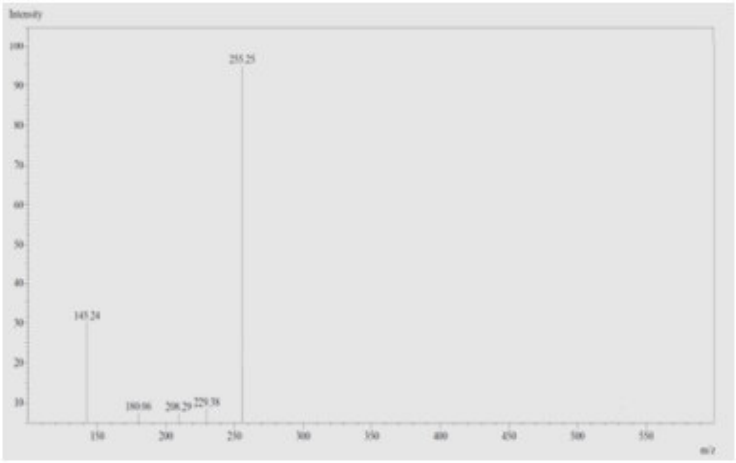

Figure 4
Mass spectrum of the purified compound

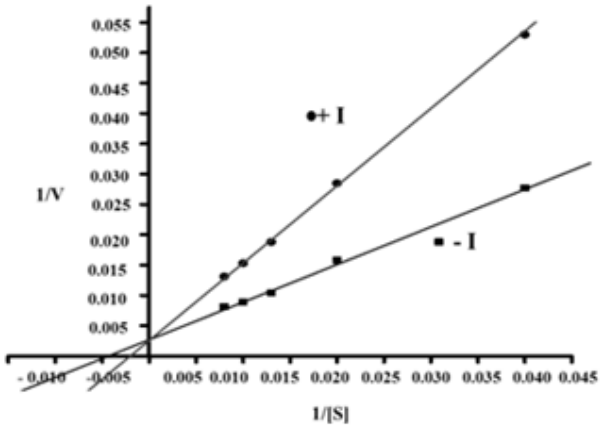

Figure 5
Lineweaver-Burk plots in the presence (+I, K_m=607.9 µM) and absence (-I, K_m=257.4 µM) of the purified compound. V_{max} is 358.4 µM min^{-1} in both the cases

CONCLUSION

A molecule that inhibits the porcine pancreatic lipase was isolated from the cultures of *S. tendae*. This is a competitive inhibitor of lower molecular mass. The IR spectrum and NMR studies will give its structural details which will be helpful for improving its inhibitory potency. Toxicity studies will give the information on its side effects which is important to know before using it for treating obesity. Nevertheless, the good solubility and the lower size of the molecule give it a good druggability character.

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CONFLICT OF INTEREST

Conflict of interest declared none

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