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ORIGINAL RESEARCH ARTICLE

Pancreatic Lipase Inhibitory And Cytotoxic Potential of A *Streptomyces* Species Isolated From Western Ghat soil, Agumbe, Karnataka, India

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ABSTRACT

The present study was undertaken to investigate pancreatic lipase inhibitory and cytotoxic activity of butanol extract of a *Streptomyces* species isolated from the western ghat soil samples of Agumbe, Karnataka, India. The isolated organism was identified on the basis of morphological, cultural and biochemical parameters. The isolate was inoculated into Starch casein broth, incubated aerobically on a rotary shaker for 7 days at 30^{o}C. After incubation, the culture broth was extracted with butanol solvent. Pancreatic lipase inhibitory (Anti-obesity) activity of solvent extract was tested against chicken pancreatic lipase using olive oil as the substrate. Cytotoxic activity was determined in terms of lethal nature of the extract towards the brine shrimp *Artemia* nauplii. The inhibition of lipase by the extract was concentration dependent and an inhibition of >60% was observed at extract concentration 50mg/ml. The mortality of brine shrimp larvae by the extract was also concentration dependent. Mortality of larvae was 100% at extract concentration 1000µg/ml. The LC_{50} of solvent extract was found to be 42.11µg/ml. The present study highlighted the pancreatic lipase inhibitory and cytotoxic potential of crude solvent extract of a *Streptomyces* species recovered from soil. The metabolites of actinomycetes from western ghat area of Karnataka may be useful as anti-obesity and cytotoxic agents. Further studies are needed to be carried out to isolate the active and characterize the active principles present in the crude solvent extract and determine their biological activity.

Key words: Western Ghats, *Streptomyces*, Pancreatic lipase, Olive oil, Brine shrimp

INTRODUCTION

Microbial metabolites are important for treatment of bacterial and fungal infections (penicillins, erythromycins, streptomycin, tetracyclines, vancomycin, and amphotericin), Cancer (doxorubicins, daunorubicin, mitomycin and bleomycin), transplant rejection (cyclosporine and rapamycin), high cholesterol (lovastatin and mevastatin) etc. There are over 23,000 known microbial secondary metabolites, 42% of which are produced by actinobacteria, 42 % by fungi, and 16 % by other bacteria. Actinomycetes, belonging to the order Actinomycetales, are a large group of Gram positive bacteria that grow as hyphae like fungi responsible for the characteristic earthy smell of freshly turned healthy soil. Actinomycetes are characterized by the formation of substrate and aerial mycelium on solid media, presence of spores and a high GC content (60-70 mol %) of the DNA. Actinomycetes are the most economically and biotechnologically valuable prokaryotes. The attention paid to this group raised notably after the discovery of streptomycin by Waksman and Schatz in 1943. They are responsible for the production of about half of the discovered bioactive secondary metabolites, notably antibiotics, antitumor agents, immunosuppressive agents and enzymes. They have provided more than half of the naturally occurring antibiotics discovered to date and continue to be screened for useful compounds. The actinomycetes especially the strains of *Streptomyces* are the most common antibiotic producing microorganisms found in soil. Out of the approximately 10,000 known antibiotics, 45-55 % are produced by streptomycetes. These have provided about two-third of naturally occurring antibiotics discovered, including many of those important in medicine, such as aminoglycosides, anthracyclines, chloramphenicol, beta lactams,

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macrolides and tetracyclines [1-11]. The Western Ghats, the range of hills running along India's west coast, are well known for their rich and unique assemblage of flora and fauna. The biological activities of microbes, in particular actinomycetes, of Western ghat soils of Agumbe, Karnataka are not much studied. Hence, the present study was carried to determine the Anti-obesity (pancreatic lipase inhibitory) and cytotoxic (Brine shrimp lethality) potential of a *Streptomyces* species isolated from Western Ghats soils of Agumbe.

MATERIALS AND METHODS

Isolation and identification of organism

The serially diluted soil sample was plated on Starch casein agar medium and incubated at 30±2°C for up to 7 days in aerobic condition. The organism was identified as a species of *Streptomyces* on the basis of morphological, staining and biochemical parameters [12,13]. The isolate was maintained on starch casein agar slant.

Fermentation and Extraction

For fermentation, a loop-full of growth of the isolate from the slant was inoculated into sterile 500ml Erlenmeyer flask containing 250ml of Starch casein broth and incubated on a rotary shaker at 200rpm for 7 days at 30°C. After incubation, the culture broth was extracted with butanol solvent and concentrated under reduced pressure to get the crude extract [14].

Pancreatic lipase Inhibitory activity of butanol extract

Extraction of lipase from Chicken (Gallus domesticus) pancreas: Pancreas of freshly slaughtered chicken were collected, washed and placed in ice cold sucrose solution (0.01M). The pancreas was homogenized in 0.01M sucrose, centrifuged; supernatant was separated and subjected to ammonium sulphate precipitation (50% saturation). The pellet obtained after centrifugation was dissolved in sucrose solution and again saturated to 50% ammonium sulphate saturation and centrifuged. The pellet obtained was dissolved in phosphate buffer and used as enzyme [15,16].

Determination of Pancreatic Lipase activity: The activity of lipase was determined by incubating an emulsion containing 8ml of olive oil, 0.4ml of phosphate buffer and 1ml of chicken pancreatic lipase for an hour on rotary shaker, followed by stopping the reaction by addition of 1.5ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH using phenolphthalein indicator [16,17].

Lipase Inhibitory activity of methanol extract: Lipase inhibitory activity of different concentrations of solvent extract was tested by mixing 100µl of each concentration of extract, 8ml of oil emulsion and 1ml of chicken pancreatic lipase followed by incubation of 60 minutes. The reaction was stopped by adding 1.5 ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH using phenolphthalein indicator [16,18]. Percentage inhibition of lipase activity was calculated using the formula: Lipase inhibition = A - B / A X 100, where A is lipase activity, B is activity of lipase when incubated with the extract.

Cytotoxic activity of butanol extract

The brine shrimp lethality test was conducted according to the method of Kekuda *et al.* [19] to determine cytotoxic nature of solvent extract. Brine shrimp *Artemia nauplii* eggs (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water which was prepared with 10g of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500ml of distilled water. After 36-48 hours, the phototropic shrimps were collected by pipette for bioassay. The different concentrations of solvent extract (10-1000µg/ml) were tested in vials containing 5ml of brine and 25 shrimp in each of three replicates. The vials were incubated at 25°C and surviving shrimps were counted after 24 hours. LC_{50} value was calculated by regression analysis. LC_{50} values greater than 1000µg/ml were considered inactive (non-toxic).

RESULTS AND DISCUSSION

The isolate was identified as a species of *Streptomyces* on the basis of microscopic, cultural and biochemical characteristics. The colony was light grey in color. Pigmentation was found to be dark green. The spores were typically arranged in open loop. The isolate was Gram positive and non-acid fast. Production of amylase, gelatinase and caseinase was detected on starch agar, nutrient gelatin and skim milk agar respectively. The isolate utilized glycerol but not sucrose and maltose. Hydrogen sulfide was not produced.

Pancreatic lipase inhibitory activity of solvent extract

Inhibitory activity of different concentrations of solvent extract was tested against chicken pancreatic lipase using olive oil as the substrate. It

was observed that the activity of lipase was drastically affected when incubated with the butanol extract. The inhibition of enzyme was found to be dose dependent i.e., inhibition of enzyme was increased on increasing the concentration of extract. An inhibition of >60% was observed at extract concentration 50mg/ml (Table 1).

Table 1: Pancreatic lipase inhibitory activity of butanol extract

Concentration (mg/ml)	Inhibition of lipase activity (%)
0.0	0.0
2.5	0.0
5.0	14.28
10.0	19.05
25.0	35.90
50.0	61.67

Cytotoxic activity of solvent extract

The cytotoxic activity of different concentrations of solvent extract, in terms of mortality of brine shrimps, is presented in Table 2. The degree of lethality of extract was directly proportional to the concentration of the extract i.e., higher the concentration, mortality was higher. Highest lethal effect was observed at 1000µg/ml extract concentration at which the mortality was 100%. The LC_{50} of butanol extract was found to be 42.11µg/ml and hence the extract is toxic. Lethal effect was lesser (20%) in case of 10µg/ml extract concentration.

Table 2: Brine shrimp lethality of butanol extract

Concentration (µg/ml)	Mortality of shrimps (%)
0.0	0.0
10.0	20.0
100.0	67.0
1000.0	100.0

One of the most important strategies in the treatment of obesity includes the development of inhibitors of nutrient digestion and absorption, in an attempt to reduce the energy intake through gastrointestinal mechanisms, without altering any central mechanisms [20,21]. Pancreatic lipase inhibition is one of the most widely studied mechanisms used to determine the potential efficacy of natural products as antiobesity agents. Orlistat, one of the two clinically approved drugs for obesity treatment, has been shown to act by inhibiting pancreatic lipase. Although it is one of the best-selling drugs worldwide, it has certain side effects such as oily stools, oily spotting, and flatulence, among others. The success of orlistat has prompted research for the identification of inhibitors that lack some of these side effects. At present, the potential of natural products for the treatment of obesity is still largely unexplored and might be an excellent alternative strategy for the development of safe and effective antiobesity drugs [22-24]. Naturally occurring compounds present an exciting opportunity for the discovery of newer anti-obesity agents. A marked dose dependent inhibition of chicken pancreatic lipase by solvent extract was observed in this study.

The brine shrimp lethality assay was proposed by Michael *et al.* [25], and later developed by Vanhaecke *et al.* [26], and Sleet and Brendel [27]. It is based on the ability to kill laboratory-cultured *Artemia* nauplii brine shrimp. It is considered to be very useful in determining various biological activities such as cytotoxic, phototoxic, pesticidal, trypanocidal, enzyme inhibition, and ion regulation activities [28-33]. The assay represents a rapid, inexpensive and simple bioassay for testing bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. It is considered as useful tool for preliminary assessment of toxicity. This method needs no special equipment and no aseptic technique. It utilizes a large number of organisms for validation, relatively small amount of sample and does not require animal serum as needed for other methods of cytotoxicity testing [34-36]. A number of studies have been carried out on cytotoxic nature of actinomycete extracts and purified metabolites on brine shrimp. Choudury *et al.* [37] showed cytotoxic activity of a brown antibiotic pigment Di- (2-ethyl hexyl)-phthalate isolated from an Actinomycetes strain. The chloroform extract as well as the isolated antibiotic showed marked cytotoxic effect against brine shrimp. From ethyl acetate extract of a *Streptomyces* strain, Sultan *et al.* [38] isolated three active metabolites from and tested their toxicity against brine shrimp. The metabolites as well as extract were found to cause a marked lethal effect on brine shrimp. Safaeian *et al.* [39] showed marked cytotoxic effect of actinomycetes isolated from Persian gulf on two *Artemia* species namely *A. urmiana* and *A. franciscana*. Manivasagan *et al.* [40] tested lethal effect of an isolated fraction BC I brine shrimp larvae [42]. In our study from the solvent extract of *Streptomyces* strain PM-32 against brine shrimp. The fraction showed strong cytotoxicity against brine shrimp. Ripa *et al.* [41] showed potent lethal effect of ethyl acetate extract and a purified compound of *Streptomyces rajshahiensis* against brine shrimp. Two compounds namely sannastatin and vicenistatin,

isolated from the cultures of *Streptomyces sannanensis*, displayed significant growth inhibitory activity against, the solvent extract caused dose dependent lethal effect on brine shrimp with LC_{50} value of 42.11µg/ml and hence the extract is toxic.

CONCLUSION

The genus *Streptomyces* is the largest producer of bioactive compounds and it remains important to discover new leader compounds from Streptomycetes for drug development. The soils of Western ghats are the richer sources for microorganisms with potent biological activities and screening programs are to be conducted to reveal the presence of pharmacologically active actinomycete isolates. In this context, the present study highlighted the pancreatic lipase inhibitory (anti-obesity) and cytotoxic potential of crude solvent extract of a *Streptomyces* species. The metabolites of actinomycetes from western ghat area of Karnataka may prove to be useful as anti-obesity and cytotoxic agents. Further studies are needed to be carried out to isolate the active and characterize the active principles present in the crude solvent extract and determine their biological activity.

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