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RESEARCH ARTICLE

ISOLATION, SCREENING OF MARINE ACTINOMYCETE STRAIN L3 FOR ANTIBACTERIAL ACTIVITY AND OPTIMIZATION OF PROCESS PARAMETERS FOR THE PRODUCTION OF LIPASE *STREPTOMYCES YOGYAKARTENSIS*

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ABSTRACT

Marine sources are increasingly being investigated as a source of microorganisms with potential to produce novel bioactive compounds and enzymes. Marine ecosystems represent a largely untapped source for isolation of new microorganisms. They are of particular interest, since they are traditionally known for their unparalleled capacity to produce biomolecules with diverse biological activities. Actinomycetes were isolated from marine sediments collected off the coast, Visakhapatnam and, Machilipatnam, Andhra Pradesh. The antibacterial and Lipase activities of the strain were determined and fermentation conditions viz., incubation time, incubation temperature, inoculum age, initial pH, were optimized for maximizing the antibacterial metabolite and enzyme production by strain L3. Physiological and Biochemical characteristics of the isolate were studied by the conventional method and identified as *Streptomyces Yogyakartensis* strain L13 by the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. Further the medium composition with respect to carbon and nitrogen sources was also optimized for increasing the antibacterial metabolite and lipase production. With the optimized conditions employed, strain showed antibacterial activity (in terms of inhibition zone diameter) of 38 mm against *Enterobacter aerogenes* & 39.5mm against *Bacillus subtilis* and Lipase activity of 41.2 U/ml.

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INTRODUCTION

Earlier the presence of indigenous marine actinomycetes in the oceans was a matter of debate; there was skepticism regarding the existence of indigenous populations of marine actinomycetes. Terrestrial actinomycetes produce resistant spores, that are known to be transported from land to sea, where they can remain dormant for many years (Bull *et al*., 2000; Cross, 1981; Good fellow and Haynes, 1984). It has been frequently assumed that actinomycetes isolated from marine samples are merely a result of these dormant spores of terrestrial origin. The supporting evidence of the existence of marine actinomycetes came from the description of Rhodococcusmarinonascene, the first marine actinomycete species to be characterized and reported (Helmke and Weyland, 1984). Actinomycetes have a profound role in the marine environment apart from antibiotic production. The degradation and turnover of various materials are a continuous process mediated by the action of a variety of microorganisms.

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There is a speculation that the increase or decrease of a particular enzyme-producing microorganism may indicate the concentration of natural substrate and conditions of the environment. The cellulolytic activity of marine actinomycetes was described by, chitinolyticactinomycetes were reported by and various industrially important enzyme producing actinomycetes have been reported (Actinomycetes are also reported to contribute to the breakdown and recycling of organic compounds. In addition, they play a significant role in mineralization of organic matter, immobilization of mineral nutrients, fixation of nitrogen, improvement of physical parameters and environmental protection. The actinomycetes are active components of marine microbial communities and form stable, persistent populations in various marine ecosystems The discovery of several new marine actinomycete taxa with unique metabolic activity in their natural environments, and their ability to form stable populations in different habitats and produce novel compounds with various biological activities clearly illustrate that indigenous marine actinomycetes indeed exist in the oceans and are an important source of novel secondary metabolites. Lipases are widely used in the processing of fats and oils,detergents and

degreasing formulations, food processing, the synthesis of fine chemicals and pharmaceuticals, paper manufacture, and production of cosmetics, and pharmaceuticals. Lipase can be used to accelerate the degradation of fatty waste and polyurethane. Most of the industrial microbial lipases are derived from fungi and bacteria. Marine sources are increasingly being investigated as a source of microorganisms with potential to produce novel bioactive compounds and enzymes. Marine ecosystems represent a largely untapped source for isolation of new microorganisms. Actinomycetes are of particular interest, since they are traditionally known for their unparalleled capacity to produce biomolecules with diverse biological activities.Actinomycetes are aerobic, gram-positive bacteria that form branching filaments or hyphae and asexual spores. They have a high guanine (G) plus Cytosine (C) content in their DNA (> 50 mol %). They have considerable practical impact because they play a major role in the mineralization of organic matter in the soil and are the primary source of most naturally synthesized antibiotics (Prescott *et al*., 2005). They are abundant in soil, and play a major role in the degradation of hydrocarbons, older plant materials, and soil humus. In addition, some actinomycetes actively degrade pesticides. Actinomycetes, primarily of the genus Streptomyces, produce an odor-causing compound called 'geosmin', which gives soils their characteristic earthy odor. They are particularly well adapted to survive in harsh environments. These are the primary source of most naturally synthesized antibiotics. These include tetracyclines, streptomycin, kanamycin, neomycin and tobramycin from various Streptomyces sp., erythromycin from Streptomyces erythraeus, vancomycin from Streptomyces orientalis, chloramphenicol from Streptomyces venezuelae and Gentamycin from Micromonospora species.

MATERIALS AND METHODS

Isolation of Actinomycetes

Study area and sampling

Marine sediment samples were collected at 1-2 feet depth with the help of sterile spatula in a sterile plastic bag from the coastal area of R.K beach, Machilipatnam district, India. The samples were immediately transferred to squeeze glass bottles and stored at 4 degree C.

Source and place of collection of samples

1. 2 meters from shore (depth – 2 feet)- R.K beach,Visakhapatnam.
2. 2 meters from shore (depth – 2 feet)-port area.
3. 2 meters from shore (depth – 2 feet)-Machillipatnam

Isolation of actinomycetes

Marine samples were stored at 4°C until isolation. Actinomycete were isolated by plating out the samples in proper dilutions. Actinomycetes colonies can often be distinguished on the plate from those of fungi and true bacteria. They are often compact, leathery giving a conical appearance and have a dry surface. About 1g of the each sample was taken into a 250ml conical flask containing 100ml of sterile water and the flasks were kept on a rotary shaker for 15 min. The suspension in each flask was serially diluted up to 10^{-5} level. Isolation was carried on Starch Casein Agar plates supplemented with rifampicin 2.5µg/mL and cycloheximide 75µg/mL to inhibit bacterial and fungal contamination respectively. The plates were seeded with a sediment sample suspension of 1.0ml each and incubated at 28°C for 14 days (Ramesh and Narayanasamy, 2009). Each day actinomycete colonies formed on the SCA plates were picked and transferred onto SCA slants and incubated at 28°C for 7 days. Only those isolates that appeared different from one another were selected. The isolates were further maintained by periodically subculturing in SCA slants. The isolates were pooled together and cultures which appeared identical to naked eye in respect of color of aerial mycelium, reverse color, soluble pigment and colony texture were eliminated. About 24 actinomycete isolates were obtained from the samples.

Primary screening by Cross-streak method

The marine actinomycete isolates were screened for antibacterial activity by cross streak method on agar plates containing starch casein agar (SCA) and nutrient agar in equal proportions. Each plate was streaked with a single isolate at the center along the diameter of the plate and incubated at 28°C for 5 days. After 5 days, test organisms were streaked perpendicular to the growth of the actinomycete culture. The intensity of inhibition produced by each isolate against the test bacteria was noted after 24 h of incubation. Plate with the same medium and without the streaking of actinomycete but with the streaking of the test organisms was maintained as a control.

Secondary screening by well diffusion method

The active isolates identified in the primary screening using cross streak method were selected for secondary screening. The active isolates were first cultivated under submerged conditions and then their ability to produce extracellular antibacterial metabolites was tested by well diffusion method (Gramer, 1976).

Submerged fermentation

The isolates were grown in an inoculation medium containing glucose (10 g/l), peptone (20 g/l), sodium chloride (5 g/l), yeast extract (5 g/l) and the pH was maintained at 7.0. The production medium consists of the inoculation medium supplemented with salt solution. The salt solution was prepared from ammonium sulphite (5 g/l), sodium hydrogen phosphate (6 g/l), potassium hydrogen phosphate (2.0 g/l), magnesium sulphate (3 g/l) and calcium chloride (3 g/l). The organism that exhibited lipolytic activity was subjected to fermentation for the production of lipase enzyme. The 72 hours old culture was prepared as spore suspension by adding 5ml sterile water. This 5ml of spore suspension was added with 45ml of inoculum medium in 250 Erlenmeyer flasks. The total contents were incubated in rotary shaker at 30℃ for 48 hours. 10% inoculum was added with 45ml of production medium. It was incubated at 30C for 7 days. At the end of 7 days fermentation, the biomass was treated with 50 ml of distilled water and stirred well for the extracellular Lipase to soluble in aqueous media. After that it was filtered by muslin cloth. Residue was again treated with 50 ml of water and filtered. The filtrate was centrifuged at 15000 rpm for 30 minutes. The clear supernatant was taken as enzyme source.

Well diffusion method

The antibacterial activity against the test bacteria was quantified by using well diffusion method on nutrient agar

medium. Nutrient agar plates were used for well diffusion method. Molten sterile nutrient agar was cooled to about 45°C, inoculated with test bacteria, mixed thoroughly, poured into sterile petriplates and allowed to settle. Wells were made in the solidified agar plates using a sterile cork borer. The clear supernatant from the fermentation broth was added to each well (50 µl) using a micropipette. The plates were kept in the refrigerator for about 2h for antibiotic diffusion and then incubated at 37°C. After 24 h, the inhibition zones were recorded.The procedure was repeated for all the promising active isolates obtained after primary screening.

Lipase activity studies

Primary Screening for Lipase activity

The tributryin agar plates were prepared and the test organisms were streaked on the agar surface. Those plates were incubated for 5 days at 37 ^{O}C. After incubation period, the lipase producing actinomycetes were screened by the formation of the clear zone. The highest zone forming actinomycetes was selected for production of lipase.

Assay for LIPASE activity

Lipase activity assay was done by titrimetric method using olive oil substrates emulsion. Add 70 ml emulsifying reagent with 30 ml olive oil and homogenized for 5 minutes. 1ml of substrate emulsion was taken in conical flask and 0.8ml of 0.2M potassium phosphate buffer (pH 7.0) was added. After that 0.2ml of enzyme was added. The total contents were incubated at 55 ℃ for 1 minute. Reaction was terminated by adding 2ml of acetone ethanol mixture (1:1 v/v). Total contents were titrated against 0.05N sodium Hydroxide using phenolphthalein as indicator.

Estimation of total protein by Lowry method

The total protein was calculated by Lowry method which has been described elsewhere. 1 Unit of lipase activity is calculated by the formula Lipase Activity = 5.61 Volume of NaOH Normality of NaOH Volume of enzyme taken Where 5.61=unit constant for identifying acid value {Unit Definition 1 Unit lipase activity = Amount of enzyme required to release 1 micro mole of fatty acid per ml per minute under above assay conditions}

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
2. Pipette out 1 mL of the sample in another test tube.
3. Make up the volume to 1 mL in all the test tubes. A tube with 1 mL of distilled water serves as the blank.
4. Now add 5 mL of reagent C to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortexing / shaking the tubes and allow to stand for 10 min.
6. Then add 0.5 mL of reagent D rapidly with immediate mixing well and incubate at room temperature in the dark for 30 min.
7. 7. Now record the absorbance at 660 nm against blank.
8. Then plot the standard curve by taking concentration of protein along X-axis and absorbance at 660 nm along Y-axis.
9. Then from this standard curve calculate the concentration of protein in the given sample.

Optimization studies

Both the physico-chemical (i.e. incubation time, incubation temperature, inoculum age, initial pH) and nutritional (i.e. addition of carbon sources, nitrogen sources) parameters are known to influence the production of microbial metabolites during a fermentation process. They were optimized by the conventional method of optimizing one independent parameter at a time, while fixing all others at a certain value. The parameter optimized in one experiment was maintained in the subsequent experiments. The same production medium used in secondary screening was used for optimization studies. In all the experiments the flasks were inoculated with 2ml of inoculum prepared from the slant cultures.

The following parameters were chosen for the optimization of Lipase production

1. Incubation time
2. Incubation temperature
3. Inoculum age
4. Initial pH
5. Carbon source
6. Nitrogen source

Optimization studies on Lipase production

Effect of Incubation time

To determine the effect of incubation time on lipase production, 50 ml of the production medium was inoculated with 4% inoculum prepared from 7day old culture and incubated at varying fermentation times ranging from 7day to 14days. After every 24 hours, the extracts were evaluated for Lipase activity.

Effect of Inoculum age

The optimum inoculum age for lipase production was determined by varying the age of inoculum from 6 day to14 day. 50 ml of the production medium was inoculated with 4% inoculum and incubated at 32 °C. From 6 th day onwords, the extracts were evaluated for lipase activity.

Effect of incubation temperature

To determine the effect of incubation temperature on lipase production, 50 ml of the production medium was inoculated with 4% inoculum prepared from 7 day old culture and incubated at varying fermentation temperatures of 28 °C, 30 °C, 32 °C, 35 °C, 36 °C,37°C ,38°C 40°C. After 7 days, the extracts were evaluated for lipase activity.

Effect of Initial pH

To determine the optimum pH lipase production, pH of the production medium was adjusted (with 0.1 N HCl and 0.1 N NaOH) to different pH values of 5, 6, 7, 8, 9 and then inoculated with 4% inoculum prepared from a 7 day old slant culture and incubated at 32 °C to 40°C for 5 days. The extracts were evaluated for lipase activity.

Effect of Carbon source

To determine the effect of carbon source on lipase production, different carbon sources such as sucrose, fructose, maltose,

starch, and glucose were added at a concentration of 1% w/v to the production medium (pH 8) and inoculated with 4% inoculum from a 7 day old culture. The flasks were incubated at 32 °C to 40°C for 5 days and subsequently evaluated for lipase activity.

Effect of Nitrogen source

To determine the effect of nitrogen source on lipase production, different nitrogen sources such as malt extract, beef extract, peptone, L-Glutamic acid and ammonium nitrate were added at a concentration of 1% w/v to the production medium (pH 8) containing glucose as the carbon source and inoculated with 4% inoculum from a 7 day old culture. The flasks were incubated at 32 °C to 40°C for 5 days and subsequently evaluated for lipase activity. Using the optimized parameters, final batch fermentation is carried out for both antibacterial metabolite and lipase produced.

RESULTS AND DISCUSSION

Isolation of actinomycetes

Marine sediment samples and the samples were collected with an aim of isolating actinomycetes. Actinomycetes were isolated by plating out the samples in proper dilutions on Starch Casein Agar (SCA) medium (supplemented with rifampicin and cycloheximide) and incubated at 28°C for 14 days. After 14 days, the actinomycete colonies were carefully isolated from the SCA plates avoiding the bacterial or fungal contamination and maintained on SCA slants.

Antibacterial activity studies

Primary screening by cross-streak method

All the actinomycete isolates were screened for antibacterial activity by cross streak method on agar plates containing starch casein agar (SCA) and nutrient agar in equal proportions. Each plate was streaked with a single isolate at the center along the diameter of the plate and incubated at 28°C for 5 days. After 5 days test organisms were streaked perpendicular to the growth of the actinomycete culture. The intensity of inhibition produced by each isolate against the test bacteria was noted after 24 h of incubation. Plate with the same medium and without the actinomycete but with the streaking of the test organisms was maintained as a control. About 24 isolates showed antibacterial activity against the test organisms used. The isolates and their antibacterial activity by cross streak method are given in Table 5.1.1

Secondary screening by well diffusion method

The 22 isolates which showed antibacterial activity against the test organisms during primary screening were further screened for extracellular antibacterial metabolite production by submerged fermentation using well diffusion method. 4% inoculum prepared from seven day old agar slant cultures of the isolates was transferred into 50 mL of production medium and incubated at 28 °C on a rotary shaker at 180 rpm for 7 days. Then the samples were collected into sterile centrifuge tubes and centrifuged at 10,000 rpm for 20 min, at 8°C and clear culture filtrate was separated. The clear supernatant was used for antibacterial assay using well diffusion method on nutrient agar plates. Wells were made in the solidified nutrient agar plates using a sterile cork borer and the 50 µL of clear supernatant was added to each well using a micropipette. The plates were kept in the refrigerator for about 2h for antibiotic diffusion and then incubated at 37°C. After 24 h, the inhibition zones were recorded. The antibacterial activities of the selected isolates during secondary screening done by using well diffusion method are given in table Isolate L3 has shown good antibacterial activity against *Bacillus subtilis* and *Enterobacter aerogenes* and has been selected for further studies.

Lipase activity studies

Primary Screening for Lipase activity

The tributryin agar plates were prepared and the test organisms were streaked on the agar surface. Those plates were incubated for 5 days at 36ºC. After incubation period, the lipase producing actinomycetes were screened by the formation of the clear zone. The highest zone forming actinomycetes was selected for production of lipase

Assay for LIPASE activity

Lipase activity assay Lipase activity assay was done by titrimetric method using olive oil substrates emulsion. Add 70 ml emulsifying reagent with 30 ml olive oil and homogenized for 5 minutes. 1ml of substrate emulsion was taken in conical flask and 0.8ml of 0.2M potassium phosphate buffer (pH 7.0) was added. After that 0.2ml of enzyme was added. Reaction was terminated by adding 2ml of acetone ethanol mixture (1:1 v/v). Total contents were titrated against 0.05N sodium Hydroxide using phenolphthalein as indicator.

Optimization studies on lipase production

Effect of incubation time

The effect of incubation time on lipase production, was studied by carrying out the fermentation at different incubation times ranging from 5 to 11days. After every 24 hours, the extracts were evaluated for lipase activity. Lipase production by strain L3 has increased steadily from day 5 to 11 and a further increase in incubation time resulted in a decrease in lipase production. The decrease in lipase production after day 8 might be due to a reduction in the available nutrients and accumulation of toxic products of metabolism.

Effect of inoculum age

The effect of inoculum age on lipase production was studied by varying the age of inoculum from5 thday to10 th day. The production medium was then inoculated with 4% inoculum, incubated at 36°C to 40°C for 5 days and the extracts were then evaluated for lipase activity.The age of the inoculum influences the growth and product formation by the microorganism. The strain L3 has shown maximum lipase production when a 4 day old inoculum was used.

Effect of incubation temperature

The effect of incubation temperature on lipase production was studied by carrying out fermentation at different temperatures viz., 28 °C, 30 °C, 32 °C, 34 °C and 36°C,37°C,38°C,40°C. The extracts of the fermentation broth were evaluated for lipase activity after 5 days.

Table 1.2. Composition of starch casein agar (SCA) medium

Constituent	Concentration (g/L)
Soluble starch	10.0
Vitamin free casein	0.3
Potassium nitrate	2.0
Sodium chloride	2.0
Dipotassiumhydrogen phosphate	2.0
Magnesium sulfate	0.05
Calcium carbonate	0.02
Ferrous sulfate	0.01
Agar	20.0
pH	7.0 ± 0.2

Table 2.1.3. Of Protein standard graph at 660nm using UV-Visible Spectrophotometer

Concentration (µg/mL)	Optical Density (660 nm)
0	0
10	0.11
20	0.17
30	0.283
40	0.413
50	0.507
60	0.614
70	0.746
80	0.81
90	0.92
100	0.984

Table 5.1.1 Antibacterial activity studies

Isolate	Gram positive			Gram negative	
	Bacillus cereus	*Bacillus subtilis*	*Staphylococcus aereus*	*Escherichia coli*	*Enterobacteraerogenes*
C1	+	+	++	-	+
C2	++	+	-	-	+
C3	+	-	-	+	++
C4	+	+	++	-	+
C5	++	++	-	+	+
C6	-	+	++	-	++
L1	+++	++	++	++	+
L2	++	-	++	-	+
L3	++	++	+++	+	+++
L4	+	-	-	+	-
L5	-	+++	++	-	-
L6	+	++	-	-	++
L7	++	+++	+	+	-
L8	++	-	-	++	++
L9	-	-	-	++	++
L10	+	-	++	++	-
M1	+	+	-	+++	+
M2	+	-	-	++	-
M3	+	-	-	+	-
M4	++	-	-	-	+
M5	++	-	-	++	++

Very good activity (+++); Good activity (++); Moderate activity (+); No activity (-)

Table 5.1.2. Antibacterial activities of the selected actinomycete isolates by well diffusion method

Isolate	Inhibition zone diameter (in mm)				
	Gram positive			Gram negative	
	Bacillus licheniformis	*Bacillus subtilis*	*Staphylococcus aereus*	*Escherichia coli*	*Enterobacteraerogenes*
C1	17	16	-	16	21
C2	22	14	16	-	-
C5	-	29	20	19	21
L1	13	-	14	-	-
L2	15	-	-	-	-
L3	17	32	33	26	36
L4	-	-	20	-	20
M1	19	20	22	12	-
M4	22	20	-	35	-
M6	36	-	29	30	19

Table 5.3.1. Effect of incubation period on lipase production

Incubation time (days)	lipase activity (U/mL)
5	10
6	12
7	20
8	18
9	16
10	14

Table 5.3.2. Effect of Inoculum age on lipase production

Inoculum age (days)	Lipase activity (U/mL)
5	15.6
6	17.31
7	19.6
8	18.21
9	17.3
10	15

Table 5.3.3. Effect of incubation temperature on lipase production

Temperature (°C)	lipase activity (U/mL)
28	9.71
30	12.85
32	14.26
34	16.92
36	18.89
37	19.45
38	15.62
40	13.58

Table 5.3.4. Effect of initial pH on lipase production

Initial pH	Lipase activity (U/mL)
5	10
6	13.4
7	16.57
8	19.71
9	14

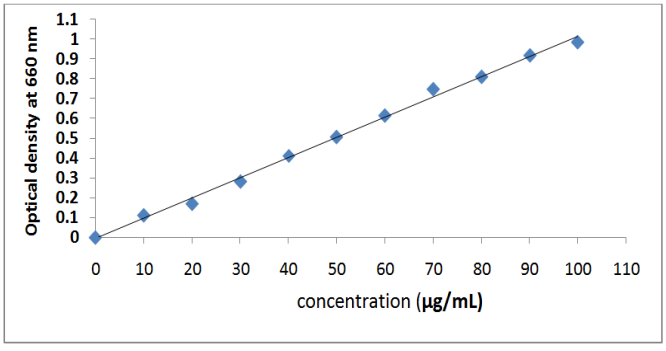
Table 5.3.5. Effect of carbon source on lipase production

Carbon source	Lipase activity (U/mL)
Sucrose	12
Fructose	17
Starch	23
Glucose	25
Maltose	17.4

Table 5.3.6. Effect of nitrogen source on lipase production

Nitrogen source	Lipase activity (U/mL)
Malt extract	12.6
Beef extract	15.8
Peptone	19.2
Ammonium nitrate	14.7
Glutamate	10.6

Optimized parameter	Optimized conditions
Incubation time	7 days
Incubation temperature	37°C
Inoculum age	7 days
Initial pH	8.0
Carbon source	Glucose
Nitrogen source	Peptone



2.1.3. Protein standard graph

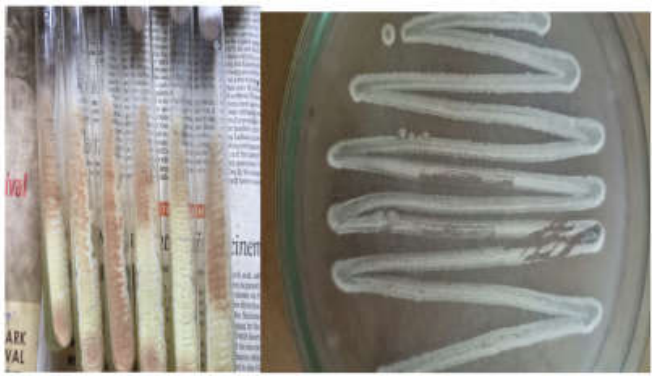


Figure 4.1 Distribution of isolated actinomycetes in various samples

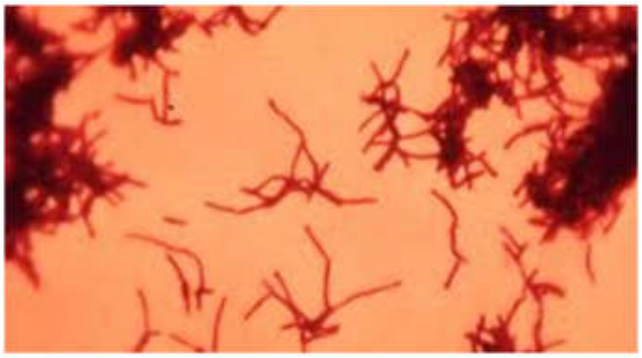


Figure 4.1.1. Microscopic view of isolated sample

Figure 5.1.2. Isolate L3

Figure 5.2.1. Tributryin Agar Plate

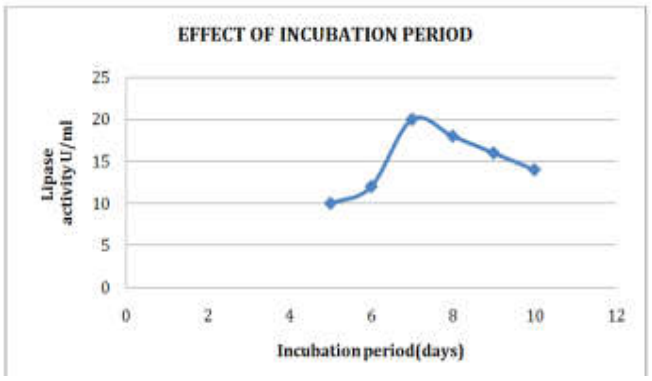


Figure 5.3.1. Effect of incubation period

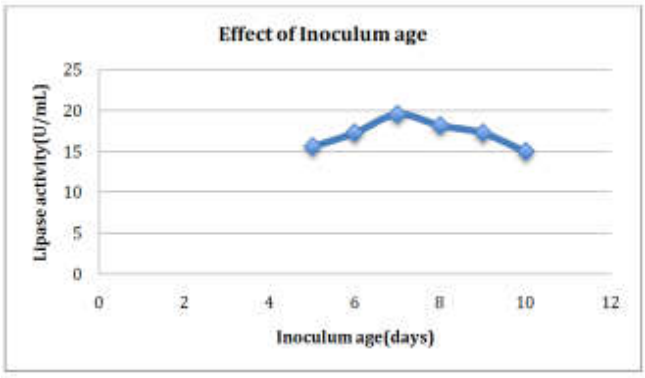


Figure 5.3.2. Effect of inoculum age

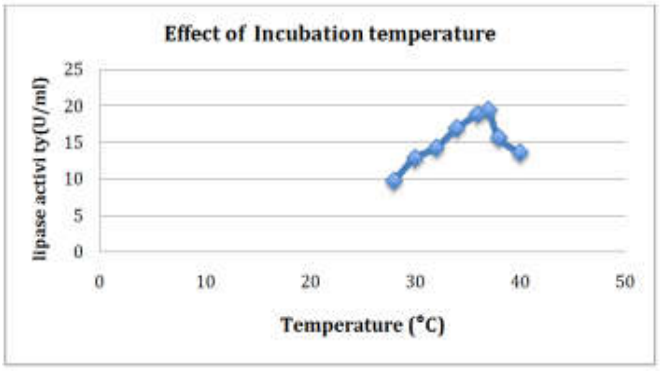


Figure 5.3.3. Effect of incubation temperature

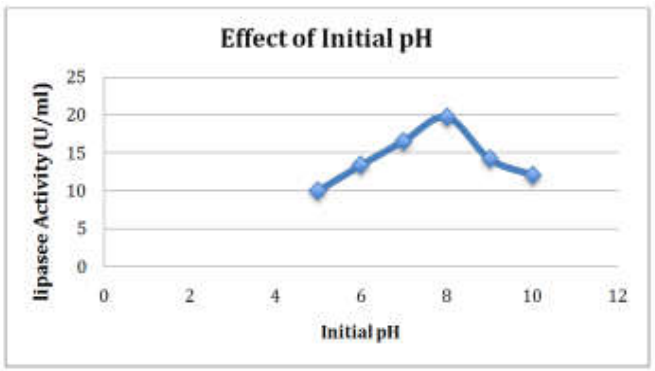


Figure 5.3.4. Effect of initial pH on lipase production

The strain L3 has shown maximum lipase production at a temperature of 37 °C From the result, we can observe that temperature had a profound impact on lipase production.

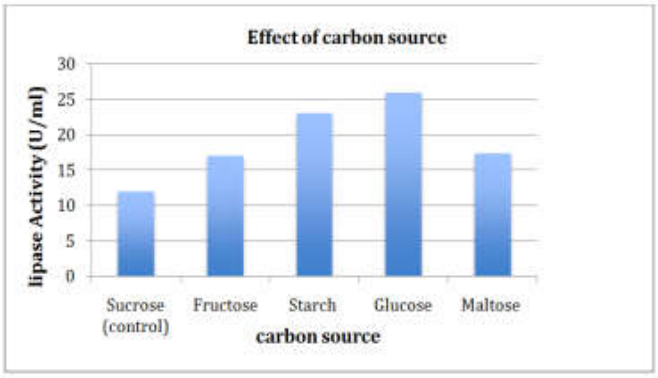


Figure 5.3.5. Effect of carbon source on lipase production

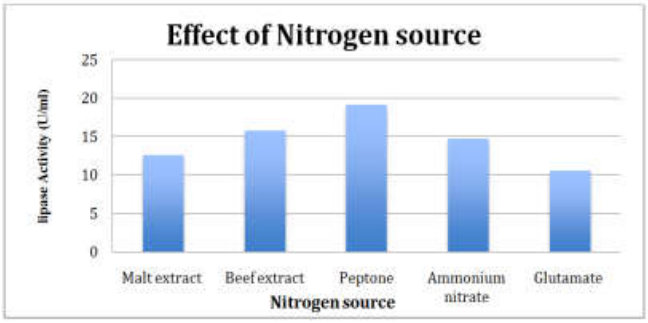


Figure 5.3.6. Effect of nitrogen source on lipase production

Effect of initial pH

To study the effect of initial pH on lipase production, pH of the production medium was adjusted to different pH values of 5.0, 6.0, 7.0, 8.0, 9.0 and then fermentation was carried out at 37 °C for 7 days. After 5 days the extracts were evaluated for lipaseactivity.Initial pH of the medium is an important factor which affects the growth and metabolite production by microorganisms during submerged fermentation. The strain L3 has shown maximum lipase production when the initial pH of the range 8.0.

Effect of Carbon source

To determine the effect of carbon source on lipase production, different carbon sources such as sucrose, starch, maltose, fructose and glucose were added at a concentration of 1% w/v to the production medium (pH 7) and inoculated with 4% inoculum, incubated at 37 °C for 7 days and subsequently evaluated for lipase activity. The strain L3 has shown maximum lipase production when glucose was used as the carbon source.

Effect of Nitrogen source

To determine the effect of nitrogen source on lipase production, different nitrogen sources such as malt extract, beef extract, peptone, L-Glutamic acid and ammonium nitrate were added at a concentration of 1% w/v to the production medium (pH 7) and inoculated with inoculum from a 7 day old culture. The flasks were incubated at 37 °C for 5 days and subsequently evaluated for lipase activity.The strain L3 has shown maximum lipase production when peptone was used as the nitrogen source. Lipase production was finally carried out with strain L3 using the optimized conditions.

Conclusion

Decrease in the rate of discovery of new compounds from terrestrial actinomycetes with an increase in the rate of re-isolation of known compounds and the rise of antibiotic resistant compounds warrant a search for actinomycetes producing novel bioactive compounds in underexplored environments. Marine and mangrove ecosystems have been an underexplored area and are potential targets for the search of novel actinomycetes. In the present study, actinomycetes were isolated from marine sediments collecte from Visakhapatnam, Machilipatnam, Andhra Pradesh. An isolate L3, Isolated from the activity against *Enterobacter aerogenes*and*Bacillussubtilis*. In addition, the Isolate L3 also exhibited lipaseproducing capability. The strain L3 was evaluated for production of antibacterial metabolite and lipase enzyme under submerged conditions. Fermentation conditions viz., incubation time, incubation temperature, inoculum age, initial pH, carbon and nitrogen sources were optimized. Morphological, biochemical and molecular characteristics were studied for the strain L3 and identified as *Streptomyces Yogyakartensis* by Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. The optimized conditions for lipase production were: incubation – 7 days; incubation temperature - 37°C; inoculum age – 4 days; initial pH – 8.0; Carbon source – glucose; Nitrogen source – peptone. With the optimized conditions employed the lipase production was 41.2 U/mL. This study shows the potential of marine sample as a source for actinomycetes with potential to produce bioactive compounds and industrial enzymes.

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