

Isolation, Screening of Mangrove Actinomycete Strains For Antibacterial Activity and Optimization of Process Parameters for the Production of Amylase

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ABSTRACT: Mangrove ecosystems are increasingly being investigated as a source of microorganisms with potential to produce novel bioactive compounds and enzymes. Actinomycetes were isolated from mangrove wetlands of Krishna estuary, Machilipatnam, Andhra Pradesh. The isolates were evaluated for antibacterial activity and strain M-2 was isolated from wetlands of Krishna estuary, Machilipatnam and Kakinada (korangi). The amylase activity of the strain were determined and fermentation conditions viz., incubation time, incubation temperature, inoculum age, initial pH, were optimized for maximizing the enzyme production by strain M-2. Further the medium composition with respect to carbon and nitrogen sources was also optimized for increasing the amylase production. With the optimized conditions employed, strain showed antibacterial activity (in terms of inhibition zone diameter) of 27 mm against *Enterobacter aerogenes* & 32 mm against *Bacillus subtilis* and amylase activity of 4.2 U/mL. It was further sent to microbial type culture collection and identification

KEYWORDS: Amylase, Submerged fermentation, Mangrove Actinomycetes, Amylase production

I. INTRODUCTION

Mangrove 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 [19]. The demand for industrial enzymes, particularly of microbial origin, is ever increasing owing to their applications in a wide variety of processes. Enzyme-mediated reactions are attractive alternatives to tedious and expensive chemical methods. Enzymes find great use in a large number of fields such as food, dairy, pharmaceutical, detergent, textile, and cosmetic industries. In the above scenario, enzymes such as proteases and amylases have dominated the world market owing to their hydrolytic reactions for proteins and carbohydrates. Amylase is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into sugar. The pancreas and salivary gland make amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

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Plants and some bacteria also produce amylase. As diastase, amylase was the first enzyme to be discovered and isolated. Specific amylase proteins are designated by different Greek letters. All amylases are glycoside hydrolases and act on α -1,4-glycosidic bonds. Amylases are used in bread making and to break down complex sugars, such as starch (found in flour), into simple sugars[28]. Yeast then feeds on these simple sugars and converts it into the waste products of alcohol and CO₂. When used as a food additive, amylase has E number E1100, and may be derived from swine pancreas or mould mushroom. Bacillary amylase is also used in clothing and dishwasher detergents to dissolve starches from fabrics and dishes. Blood serum amylase may be measured for purposes of medical diagnosis. A higher than normal concentration may reflect one of several medical conditions, including acute inflammation of the pancreas (it may be measured concurrently with the more specific lipase) but also perforated peptic ulcer, torsion of an ovarian cyst, strangulation, ileus, mesenteric ischemia, macroamylasemia and mumps. Amylase may be measured in other body fluids, including urine and peritoneal fluid. In molecular biology, the presence of amylase can serve as an additional method of selecting for successful integration of a reporter construct in addition to antibiotic resistance. As reporter genes are flanked by homologous regions of the structural gene for amylase, successful integration will disrupt the amylase gene and prevent starch degradation, which is easily detectable through iodine staining. Amylases are used for the preparation of sizing agents in textile industry, preparation of starch sizing pastes for use in paper industry, chocolate, corn syrup and removal of spots in laundry. The conversion of starch to high fructose syrup utilizes amylases, i.e in producing sweeteners. The above quality makes the future interest for the scientists to work in the amylase. This work was also an attempt towards the same goal. As strong belief is that, the goal can be achieved by identifying new strains by means of isolation and screening from various substrates and performing the optimization of the production parameters.

II. MATERIALS AND METHODS

1. Isolation of actinomycetes:

1.1 Study area and sampling:

Marine sediment samples were collected from 2 different areas. The mangrove wetlands of Krishna estuary, Andhra Pradesh. Mangrove sediment samples were collected from the two sampling sites 1. Kakinada 2. Machilipatnam. Due to extensive shipping activities and crude oil pollution, these mangroves were selected as priority sites for the study. The mangrove sediment samples were collected with a soil corer during low tides and transferred to a pre-cleaned polycarbonate glass bottles stored at -20°C and kept frozen for further analysis.

Source and place of collection of samples:

- A. Mangrove wetlands of Eastern estuary from Kakinada (Korangi), Andhra Pradesh, India.
- B. Mangrove wetlands of Krishna estuary from Machilipatnam, Andhra Pradesh, India.

1.2. Isolation of actinomycetes:

Mangrove sediment samples were stored at 4°C until isolation. Actinomycetes 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

After applying all these 4 steps, we get a filtered image that contains only text regions 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. After 14 days, 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 sub culturing in SCA slants. The composition of starch casein agar (SCA) medium contains Soluble starch(10 g/L), Vitamin free casein (0.3 g/L), Potassium nitrate (2.0 g/L), Sodium chloride(2.0 g/L), Dipotassium hydrogen phosphate (2.0 g/L), Magnesium sulfate (0.05 g/L), Calcium carbonate (0.02 g/L), Ferrous sulfate (0.01 g/L), Agar (20.0 g/L), pH7.0 ± 0.2. The isolates were pooled together and cultures which appeared identical to naked eye in respect of color of aerial mycelium, reverse color,

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soluble pigment and colony texture were eliminated. About 24 actinomycete isolates were obtained from the samples[35].

Primary screening by Cross-streak method:

The mangrove 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.

Submerged fermentation:

Well sporulated slants of the isolates (7 days old) were used for antibacterial metabolite production studies. 2 ml of sterile water was transferred aseptically into each slant and the growth of the isolate on the surface of the medium was scrapped with a sterile inoculating needle and transferred into 50 ml of production medium contains Sucrose 20.0(g/L), Malt extract 10.0(g/L), Yeast extract 4.0(g/L), Dipotassium hydrogen phosphate 5.0(g/L), Sodium chloride 2.5(g/L), Zinc sulphate 0.04(g/L), Calcium carbonate 0.4(g/L), pH 7.0 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.

Primary Screening for Amylase activity:

Nutrient agar plates with 1% soluble starch were used for primary screening for amylase activity. Each plate was inoculated with actinomycetes as a small spots and incubated at 37°C for 5days. After 5 days the plates were flooded with 1% I and KI solution.

A clear zone around colony against dark blue background indicates amylase activity.

Assay for Amylase Activity:

The reaction mixture containing 1ml of 1% (w/v) starch solution and 1 ml of enzyme extract were incubated at room temperature for 15 minutes. The reaction was terminated by the addition of 2ml Dinitrosalicylic (DNS) reagent and tubes kept at zero time incubation served as control. The solution was heated in a boiling water bath for 5 minutes followed by the addition of 1ml potassium sodium tartarate solution. After cooling, the volume of the solution was made up to 10ml and optical density was read at 560 nm. One amylase unit equals to that amount of enzyme needed to release 1mg of reducing sugar (maltose as standard) for 15 minutes at 37°C. [

DNS Reagent Preparation:

About 1g of DNS is dissolved in 50ml of distilled water.To this solution add about 20g of sodium potassium tartarate, the solution turns milky yellow in colour then add 20ml of 1.6g of NaOH, which turns the solution to transparent orange yellow colour. The final volume is made to 100ml with the distilled water. This solution is stored in an amber coloured bottle[35].

180gm of maltose is weighed and added to 100ml distilled water.0.1 to 1 mg/ml concentrations of maltose standard solution was taken into different test tubes and made up to 1 ml with distilled water.2 ml of Dinitrosalicylic (DNS) reagent was added and incubated for 5min at 95 to 100C.1ml of Sodium potassium tartarate is added. The absorbance was read at 560nm using UV-Visible Spectrophotometer (Table 1.1) and graph (a) maltose standard graph.

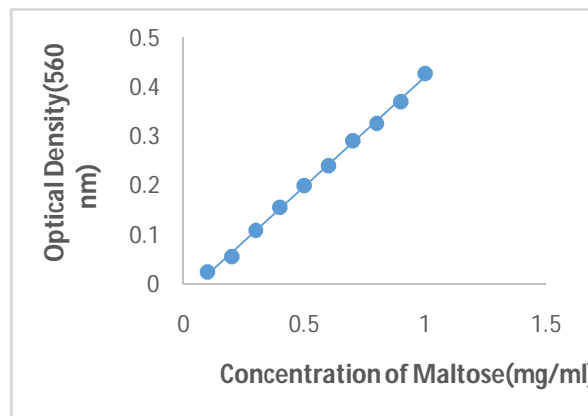
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Concentration of Maltose(mg/ml)	Optical Density(560 nm)
0.1	0.025
0.2	0.056
0.3	0.110
0.4	0.156
0.5	0.2
0.6	0.241
0.7	0.291
0.8	0.327
0.9	0.371
1.0	0.428



(a) Maltose standard graph

(1.1) Table of Maltose standard graph at 560nm using UV-Visible Spectrophotometer

The present study deals with the isolation of actinomycetes with potential to produce novel bioactive compounds and enzymes. The potential isolate was identified and the fermentation conditions for the production of antibacterial metabolite are optimized.

III. RESULTS

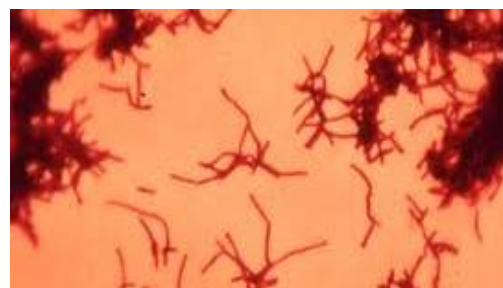
Isolation of Actinomycetes:

Samples from Coringa mangrove forest 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

Figure: (b) Actinomycetes isolated from mangrove samples using SCA medium and Figure: (c) Microscopic view of isolated sample.



(b) Distribution of isolated actinomycetes in various samples.



(c.) Microscopic view of isolated sample

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<i>Sample Series</i>	<i>Number of isolates obtained</i>	<i>Designation of the isolates</i>
L	7	L1 – L7
M	7	M1 – M7

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 centre 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 no: 1.2
Table: 1.2 Antibacterial activity of actinomycete isolates by cross streak method[13].

<i>Isolate</i>	<i>Gram positive</i>			<i>Gram negative</i>	
	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Enterobacteraerogenes</i>
L1	+	+	-	+	+
L2	++	+	+	-	-
L3	-	+	+	+	++
L4	+	-	++	-	+
L5	++	++	-	+	+
L6	-	+	++	-	++
L7	+++	++	++	++	+
M1	++	-	-	-	-
M2	++	++	+++	+	+++
M3	-	-	+	-	+
M4	+	+++	++	+	-
M5	+	++	-	-	++
M6	++	+++	+	+	-
M7	++	-	-	++	++
Very good activity (+++); Good activity (++); Moderate activity (+); No activity (-)					

Table: 1.2 Antibacterial activity of actinomycete isolates by cross streak method

Secondary screening by well diffusion method:

The 21 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.

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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 no: 1.3.

Table No: 1.3 Antibacterial activities of the selected actinomycete isolates by well diffusion method.

Isolate	Inhibition zone diameter (in mm)				
	Gram positive			Gram negative	
	<i>Bacillus licheniformis</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Enterobacteraerogenes</i>
L1	17	16	-	16	21
L2	22	14	16	-	-
L3	-	29	20	19	21
L4	13	-	14	-	-
M1	15	-	-	-	-
M2	17	32	33	26	27
M3	-	-	20	-	20
M4	19	20	22	12	-

Isolate M-2 has shown good antibacterial activity against *Bacillus subtilis* and *enterobacteraerogenes* and has been selected for further studies.

Optimization studies on Amylase production:

(i.) **Effect of incubation time:** To determine the amylase production, the production medium was inoculated with 7 day old culture of strain M-2 and incubated at 30°C, 180 rpm, for 11 days. For every 24 hours, the fermentation broth was centrifuged at 4°C and 10,000 rpm for 20 minutes. The extracts were tested for amylase activity by UV-Spectrophotometer at 560 nm. The absorbance values were analyzed using maltose standard graph. Maximum amylase activity was observed on 8th hour of Day-8 (Table 1.4 and Figure (d)).

Incubation time (days)	Amylase activity (U/mL)
1	0.25
2	0.4
3	0.75
4	1.2
5	1.65
6	2.15
7	2.55
8	2.75
9	2.15
10	1.6
11	1.1

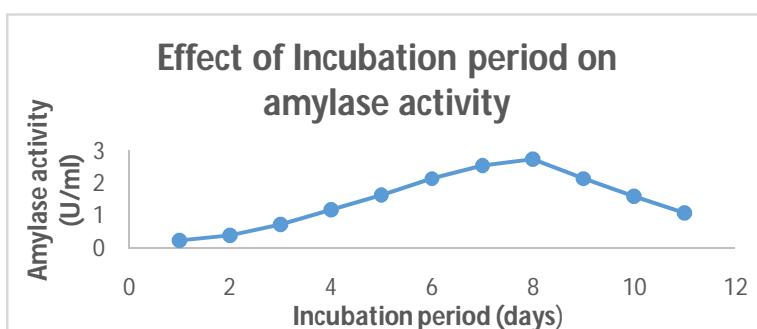


Figure:(d.)Effect of incubation period on amylase production

Table 1.4: Effect of incubation period on amylase production.

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(ii.) Effect of incubation temperature:

The effect of incubation temperature on amylase production was studied by carrying out fermentation at different temperatures viz., 28 °C, 30 °C, 32 °C, 34 °C and 36°C. The extracts of the fermentation broth were evaluated for amylase activity after 3 days. The strain M-2 has shown maximum amylase production at a temperature of 30°C (Table 1.5 and Figure (e.)). From the result, we can observe that temperature had a profound impact on amylase production.

Temperature (°C)	Amylase activity (U/mL)
26	1.9
28	2.6
30	3
32	2.55
34	1.92

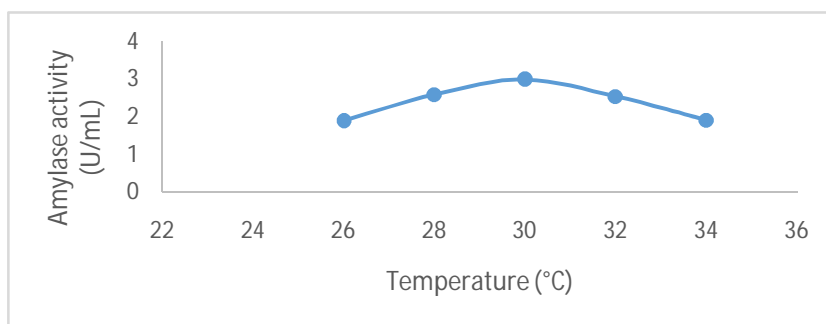


Figure (e.) Effect of incubation temperature on amylase production

Table: 1.5 Effect of incubation temperature on amylase production.

(iii.) Effect of initial pH:

The pH of the production medium was adjusted to different pHs (5, 6, 6.5, 7, 7.5, 8, 9, 10) with 0.1N NaOH/0.1N HCl. All the flasks were inoculated with the strain M-2 of the actinomycete culture and incubated at 30°C, 180 rpm for 8 hour. After incubation, the extracts were analysed for amylase activity by UV-Spectrophotometer at 560 nm. The absorbance values were analyzed using maltose standard graph. Maximum amylase activity was observed at pH-7 (Table 1.6 and Figure (gf.)).

Initial pH	Amylase activity (U/mL)
5.0	1.3
6.0	2.1
6.5	2.55
7.0	2.9
7.5	2.6
8.0	2.4
9.0	1.9

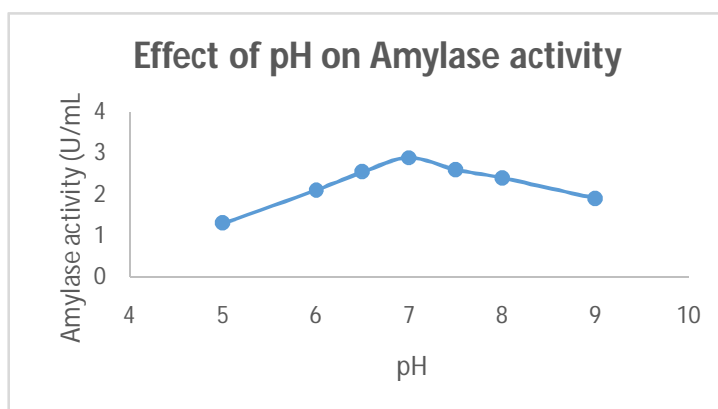


Figure (f.): Effect of initial pH on amylase activity

Table: 1.6 Effect of initial pH on amylase activity

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(iv.) Effect of Carbon source:

To determine the effect of carbon source on amylase production, different carbon sources such as sucrose, starch, maltose, lactose and glucose were added at a concentration of 1% w/v to the production medium (pH 7) and inoculated with 4% inoculum, incubated at 30°C for 3 days and subsequently evaluated for amylase activity.

The strain M-2 has shown maximum amylase production when sucrose was used as the carbon source (Table 1.7 and Figure (g.)).

<i>Carbon source</i>	<i>Amylase activity (U/mL)</i>
Glucose	2.7
Lactose	0.95
Maltose	2.65
Starch	2.5
Sucrose	3.15

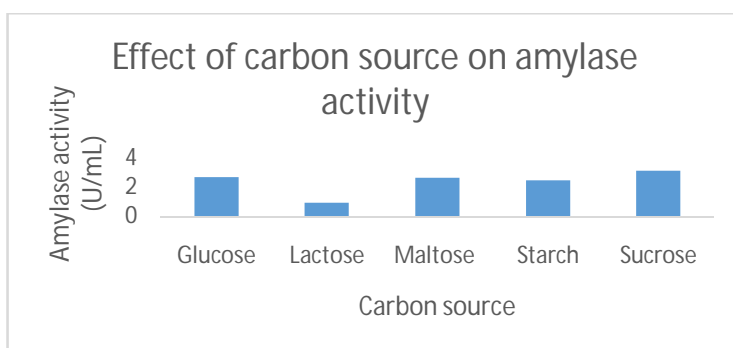


Table: 1.7 Effect of initial carbon source on amylase activity

Figure (g.): Effect of initial pH on amylase activity

(v.) Effect of Nitrogen source:

To determine the effect of nitrogen source on amylase production, different nitrogen sources such as Malt Extract, Beef Extract, Yeast Extract, Peptone and Ammonium Nitrate were tested. Each of them at a concentration of 0.3g/l with other optimized conditions was supplemented.

The strain M-2 has shown maximum amylase production when Ammonium Nitrate was used as the nitrogen source (Table 1.8 and Figure (h.)).

<i>Nitrogen source</i>	<i>Amylase activity (U/mL)</i>
Malt extract	0.45
Beef extract	2.9
Yeast extract	2.4
Ammonium nitrate	4.2
Peptone	3.1

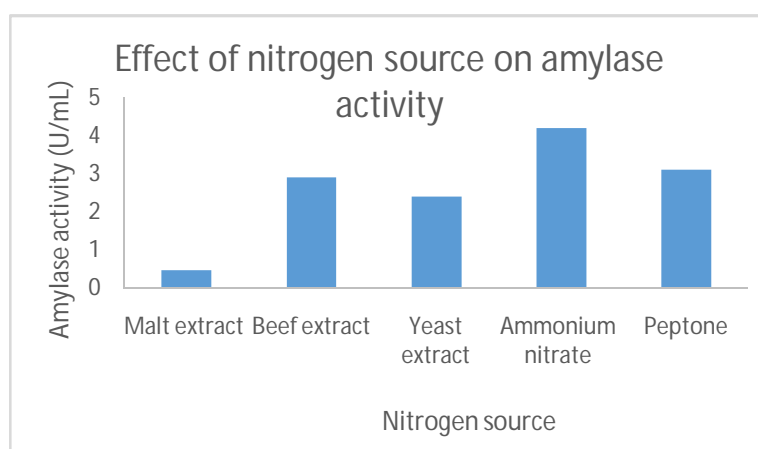


Table: 1.8 Effect of nitrogen source on amylase production

Figure (h.) Effect of nitrogen source on amylase production

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Amylase production was finally carried out with strain M-2 using the optimized conditions.

Optimized conditions for amylase production.

Optimized parameter	Optimized conditions
Incubation time	8 days
Incubation temperature	30 °C
Initial pH	7.0
Carbon source	Sucrose
Nitrogen source	Ammonium nitrate

With the optimized conditions employed, strain M-2 showed amylase activity of **4.2 U/mL**.

IV. 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 mangrove sediments. Samples collected from mangrove wetlands of Krishna estuary, Machilipatnam, and Kakinada (korangi) Andhra Pradesh. An isolate M-2, Isolated from the mangrove wetlands of Krishna estuary showed antibacterial activity against *Enterobacter aerogenes* and *Bacillus subtilis*. In addition, the Isolate M-2 also exhibited amylase producing capability.

The strain M-2 was evaluated for production of amylase enzyme under submerged conditions. Fermentation conditions viz., incubation time, incubation temperature, inoculum age, initial pH, carbon and nitrogen sources were optimized.

With the optimized conditions employed, the zone of inhibition against *Enterobacter aerogenes* and *Bacillus subtilis* were 27 and 32 mm respectively.

The optimized conditions for amylase production were: incubation – 8 days; incubation temperature - 30°C; initial pH – 7.0; Carbon source – Sucrose, Nitrogen source – Ammonium nitrate. With the optimized conditions employed the amylase production was 4.2 U/mL.

This study shows the potential of mangrove ecosystems as a source for actinomycetes with potential to produce bioactive compounds and industrial enzymes.

REFERENCES

- [1] Abraham E.P., and Newton G.G.F., 1960. Chemistry and classification of antibiotics. *Brit. Med. Bull.*, 16: 3-10.
- [2] Adinarayana G., Venkateshan M.R., Bapiraju V.V., Sujatha P., Premkumar J., Ellaiah P. and Zeeck A, 2006. Cytotoxic compounds from the marine actinobacterium. *Bio. Org. Khim.*, 32: 328-334.
- [3] Aharonowitz Y., 1980. Nitrogen metabolite regulation of antibiotic synthesis. *Ann. Rev. Microbiol.*, 34: 209-233.
- [4] Alan T. Bull and James E.M. Stach, 2007. Marine actinobacteria: new opportunities for natural product search and discovery. *Trends in Microbiology*, 15: 491-499.
- [5] Alavandi S. V., 1990. Relationship between heterotrophic bacteria and suspended particulate matter in the Arabian Sea (Cochin). *Indian J. Mar. Sci.*, 30: 89-92.
- [6] Allrege A.L. and Silver M.W., 1988. Characteristics, dynamics and significance of marine snow. *Prog. Oceanog.*, 20: 41-82.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

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- [7] Alongi D.M., Boto K.G., Robertson A.I., 1992. Nitrogen and Phosphorus cycles in tropical mangrove ecosystems. Washington DC: Am Geophys. Univ., 41:251–292.
- [8] Alongi D.M., Christoffersen P. and Tirendi F., 1993. The influence of forest type on microbial-nutrient relationships in tropical mangrove sediments. *J. Exp. Mar Biol. Ecol.*, 171: 201-223.
- [9] Alongi D.M., 1988. Bacterial productivity and microbial biomass in tropical mangrove sediments. *Microb. Ecol.*, 15: 59-79.
- [10] Anderson A.S., Wellington E.M.H., 2001. The taxonomy of *Streptomyces* and related genera. *Int. J. Syst. Evol. Microbiol.*, 51: 797–814.
- [11] Balachandran C., Duraipandiyar V. and Ignacimuthu S., 2012. Purification and characterization of amylase enzyme from actinomycetes and its cytotoxic effect on cancer cell line (A549). *Asian Pacific Journal of Tropical Biomedicine*, S392-S400.
- [12] Baldacci E., 1962. The classification of actinomycetes in relation to their antibiotic activity. *Advan. Appl. Microbiol.*, 3: 257-278.
- [13] Bartz Q.R., Elder C.C., Frohaedt R.P., Fusari S.A. Haskell T.H., Johannessen D.W. and Ryder A., 1954. Azaserine, a new tumor-inhibitory substance. Isolation and characterization of azaserine. *Nature*. 173: 74.
- [14] Bashar H., 2010. The antibiotics market (from the analysts cough). *Drug discovery, Nat Rev*, 9: 675-676.
- [15] Bano N., Nisa M.U., Khan N., Saleem M., Harrison P.J., Ahmed S.I., Azam F., 1997. Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River Delta, Pakistan. *Mar. Ecol. Prog. Ser.*, 157: 1-12.
- [16] Beulow G.H. and Johnson M.J., 1952. Effect of separation on citric acid production in 50 gallon tanks. *Ind. Eng. Chem.*, 44: 2945-2946.
- [17] Bijender K. Bajaj and Priyanka Sharma, 2011. An alkali-thermotolerant extracellular amylase from a newly isolated *Streptomyces* sp. DP2. *New Biotechnology*, 28: 725 – 732.
- [18] Chun-Fai Yua, Peter Hoi-Fu Yua, Pui-Ling Chana, Qun Yana and Po-Keung Wong, 2004. Two novel species of tetrodotoxin-producing bacteria isolated from toxic marine puffer fishes. *Toxicon*, 44: 641–647.
- [19] Colquhoun J. A., Mexson J., Goodfellow M., Ward A. C., Horikoshi K. and Bull A. T., 1998. Novel rhodococci and other mycolata actinomycetes from the deep sea. *Antonie van Leeuwenhoek*, 74: 27–40.
- [20] Cross T., 1981. Aquatic actinomycetes: a critical survey of the occurrence, growth and role of actinomycetes in aquatic habitats. *J Appl. Bacteriol.*, 50: 397-423.
- [21] Crout D.H., Macmanus D.A., Rica J.M., Singh S., Critchley P. and Gibson W.T., 1992. Biotransformation in the peptide and carbohydrate fields. *Pure & Appl. Chem.*, 64: 1079-1084.
- [22] Dar S.A., Kleerebezem R., Stams A.J.M., Kuenen J.G., Muyzer G., 2008. Competition and coexistence of sulfate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. *Appl. Environ. Microbiol.*, 78: 1045-1055.
- [23] Debananda S. Ningthoujam, PintubalaKshetri, SuchitraSanasam and Salam Nimaichand, 2009. Screening, identification of best producers and optimization of extracellular amylases from moderately halophilic alkali-thermotolerant indigenous actinomycetes. *World Applied Sciences Journal*, 7: 907-916.
- [24] De Azeredo L.A.I., Freire D.M.G., Soares R.M.A., Leite S.G.F. and Coelho R.R.R., 2004. Production and partial characterization of thermophilic amylases from *Streptomyces* sp. isolated from Brazilian cerrado soil. *Enzyme and Microbial Technology*, 34: 354–358.
- [25] Dhanasekaran D., Selvamani S., Panneerselvam A. and Thajuddin N., 2008. Isolation and characterization of actinomycetes in Vellar Estuary, Annagkoil, Tamil Nadu. *African Journal of Biotechnology*, 8: 4159-4162.
- [26] Dias A.C.F., Andreote F.D., Dini-Andreote F., Lacava P.T., Sa A.L.B., Melo I.S., Azevedo J.L., Araujo W.L., 2009. Diversity and biotechnological potential of culturable bacteria from Brazilian mangrove sediment. *World J. Microbiol. Biotechnol.*, 25: 1305-1311.
- [27] Eccleston G.P., Brooks P.R., Kurtböke D.I., 2008. The occurrence of bioactive Micromonosporae in aquatic habitats of the Sunshine Coast in Australia. *Marine Drugs*, 6: 243-261.
- [28] Fenical W., 2006. Marine pharmaceuticals: past, present, and future. *Oceanography*, 19: 110-119.
- [29] Fenical W. and Jensen P.R., 2006. Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.*, 2: 666-673.
- [30] Fortune W.B., McCormick S.L., Rhodehamel H.W. and Stefaniak J.J., 1950. Antibiotics development. *Ind. Eng. Chem.*, 42: 191-198.
- [31] Gan Jun Yuan, Kui Hong, Hai Peng Lin and Jia Li, 2010. Azalomycin F4a 2-ethylpentyl ester, a new macrocyclic lactone, from mangrove actinomycete *Streptomyces* sp. 211726. *Chinese Chemical Letters*, 21: 947–950.
- [32] Sathiyaseelan K. and Stella D., 2011. Isolation, identification and antimicrobial activity of marine actinomycetes isolated from Parangipettai. *Recent Research in Science and Technology*, 3: 74-77.
- [33] Saxena D., Loka-Bharathi P.A. and Chandramohan D., 1988. Sulfate reducing bacteria from mangrove swamps of Goa, central west coast of India. *Indian J Mar. Sci.*, 17: 153-157.
- [34] Sengupta A. and Chaudhuri S., 2002. Arbuscularmycorrhizal relations of mangrove plant community at the Ganges river estuary in India. *Mycorrhiza*, 12: 169-174.
- [35] Vijayalakshmi, Sushma, K., Abha, S. and Chander, P. 2012. Isolation and characterization of *Bacillus subtilis* KC3 for Amylolytic activity. *International Journal of Bioscience, Biochemistry and Bioinformatics*. 2(5): 336-341.
- [36] Vidyalakshmi, R., Paranthaman, R. and Indhumathi, J. 2009. Amylase production on Submerged fermentation by *Bacillus* sp. W. *J. Chem.*4(1): 89-91.
- [37] Vaseekaran, S., Balakumar, S. and Arasaratnam, V. 2010. Isolation and identification of a bacterial strain producing thermostable α - Amylase, *Tropical Agricultural Research*. 22 (1): 1 -11..