

Isolation and Screening of *Actinomycetes* from Mangrove Soil for Enzyme Production and Antimicrobial Activity

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Abstract— Mangrove ecosystems are the most productive ecosystems, and possess unique environmental conditions, but are least explored. This study was carried out to isolate *Actinomycetes* strains that have potential to produce commercially important enzymes as well as antibiotics from least explored mangrove soils. The soil sample was collected from Dumas – Bhimpore mangrove region, Surat. Using dilution plate technique three isolates were obtained and were characterized based on morphological, physiological and biochemical parameters. Screening was done for four important enzymes such as amylase, cellulase, L-asparaginase and protease production and respective enzymatic activities by isolated strains. All strains were able to produce amylase enzyme. Strain 1 was found to have potential to produce amylase and cellulase enzymes; strain 2 was found to have potential to produce amylase, and L-asparaginase enzymes while strain 3 was found to have potential to produce all four enzymes. Isolates were also screened for antimicrobial activity against test organisms such as *Salmonella typhimurium*, *Serratia marcescens*, *Bacillus cereus*, *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* and antifungal activity against *Rhizopus*, *Penicillium*, *Neurospora crassa*, *Aspergillus niger* and *Fusarium oxysporum*. Strain 1 was found to have no potential antimicrobial activity against any test bacteria. No isolate was found to have potential antifungal activity against test fungus.

Keywords— *Actinomycetes*, Mangrove ecosystem, Enzymatic activity, Antimicrobial and Antifungal activity.

I. INTRODUCTION

Mangroves are the unique woody plants of intertidal coasts situated in tropical and subtropical zones. Mangrove forests are among the world's most productive ecosystems. They are adapted to survive in harsh conditions such as high salinity, high temperature, low oxygen, extreme tides, muddy and anaerobic soil, and forceful winds. The mangroves biomass is greater than any other aquatic systems. Mangroves prevent soil erosion and deposition of silt. Global mangroves have an estimated cover of 15.2 million hectares (FAO, 2007). Marine environments are largely untapped source for the isolation of new microorganisms with potentiality to produce active secondary metabolites [1]. Among such microorganisms, *Actinomycetes* are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities [2]. Multiple antibiotic resistant pathogens are rapidly

emerging and hence there is a constant demand for new antibiotics in market. The name "*Actinomycetes*" was derived from Greek word "atkis" (a ray), and "mykes" (fungus) and has the features of both bacteria and fungi [3].

Taxonomically, *Actinomycetes* are clubbed with bacteria in same class of schizomycetes but confined to order Actinomycetales [4]. *Actinomycetes* are Gram positive with high G+C content in their DNA. *Actinomycetes* are numerous and widely distributed in soil. They are heterotrophic, aerobic and mesophilic (25-30°C) and some species are thermophilic growing at temperature 55-65°C e.g. *Thermoactinomycetes*, *Streptomyces*. *Actinomycetes* possess various unique and interesting features. They are of great importance as antibiotic producers and also other therapeutically significant compounds. Recently, the marine derived *Actinomycetes* are recognized as a source of novel antibiotic and anticancer agent with unusual structure and properties [5]. In the present study, an attempt was made to isolate and screen the *Actinomycetes* from the mangrove soil of Dumas-Bhimpore region, which can be valuable resource of various enzymes and antibiotic production.

II. MATERIALS AND METHODS

A. Sample Collection

The soil samples were collected during the month of December 2015 from Dumas Bhimpore Surat mangrove region. Black, moist and sandy textured soil around mangrove plants was preferred for experiment. Using sterile spatula top soil (4 — 5 cm) was taken out and transferred to sterile polythene bag. Top soil was used because here most of the microbial activity takes place, and thus most of the bacterial population is concentrated in this layer. Part of soil sample was air dried at room temperature and part was sun-dried for ten days, this helps to decrease the population of Gram negative bacteria.

B. Isolation of *Actinomycetes*

1g of air dried and sun dried soil samples each were added in sterile 100 mL of 0.85% Normal Saline. These flasks were kept on orbital shaker at 200 rpm for 30 minutes at 28°C. Two methods of isolation were used 1) Direct Streak Method and 2) Dilution Plate Method. Five media reported in literature

were used for isolation of strains. They were: ISP-2 (International *Streptomyces* Project type-2), Glucose Asparagine Agar, Starch Casein Agar, Potato Dextrose Agar, and Nutrient Agar. Media were supplemented with antibiotics amphotericin B (50 µg/mL) and rifampicin (50 µg/mL) to inhibit the fungal and bacterial contamination respectively. Media without antibiotics was also used for isolation. After inoculation plates were incubated in incubator at 30°C for 3 – 4 days. After incubation, *Actinomyces* colonies were selected and maintained by subculturing on ISP-2 agar and Nutrient Agar slants stored at 4°C for further use.

C. Identification of *Actinomyces* Isolates

Morphological characterization which includes colony characteristics and aerial mass colour was performed. Physiological parameters like pH (5.0, 7.0, 9.0); temperature (30°C, 40°C, 50°C); NaCl concentration (5%, 10%, 15%) and Biochemical tests such as starch hydrolysis and sugar (Dextrose, Sucrose, Xylose, Raffinose, Mannose, Cellulose, Rhamnose, Inositol, Maltose, Lactose and Fructose) utilization tests were performed.

D. Screening of *Actinomyces* for Enzyme Production

The *Actinomyces* strains isolated were screened for presence of different enzymes such as Amylase, Cellulase, L-asparaginase, and Proteases.

1) *Primary Screening for Amylase Production*: The strains isolated were inoculated on Starch agar media and incubated at 30°C in an incubator for 3 – 4 days. After incubation the plates were flooded with Gram's iodine solution. It was allowed to stand for few minutes and poured off. Clear distinct zone around the amylase producing colonies were observed.

1.1) *Amylase Assay*: The amylase producing positive strain obtained through primary screening was inoculated in 50 mL of starch broth media and incubated in orbital shaking incubator at 150 rpm at 30° C for 48 – 72 hrs. After incubation the broth was collected aseptically in sterile centrifuge tubes and centrifuged at 5000 rpm for 30 minutes at 4°C in refrigerated centrifuge. The cell free supernatant (crude enzyme extract) was collected and enzymatic assay was performed in duplicates. 1 mL of 1% starch substrate was added to each 0.2 mL, 0.3 mL, 0.4 mL, 0.6 mL and 1 mL aliquots of enzyme solution; final volume upto 3 mL was made up with distilled water. Blank was prepared without addition of enzyme solution. The reaction mixture was incubated in waterbath at 37° C for 20 minutes. The reaction was stopped by addition of 1 mL DNS reagent and kept in boiling waterbath for 15 minutes. Final volume upto 10 mL was made up with distilled water after mixture is cooled to room temperature. Absorbance was taken at 540 nm. Standard of maltose (1 mg/mL) was prepared using DNS method. 1 unit (IU) is defined as the amount of enzyme that released 1µmol of maltose from starch per minute at pH 7.5 at 37°C.

2) *Primary Screening for Cellulase Production*: The strains isolated were inoculated on Czepak mineral salt agar media

and incubated at 30° C in an incubator for 3 – 4 days. After incubation the plates were flooded with 0.5% Congo red solution and allowed to stand for 10 minutes, then the solution was decanted, excess stain was removed using 1 M NaCl solution as destainer and allowed to destain for 10 – 15 minutes. Clear yellow zone around the cellulase producing colonies were observed.

2.1) *Cellulase Assay*: The Cellulase producing positive strain obtained through primary screening was inoculated in 50 mL of Czepak mineral salt broth media and incubated in orbital shaking incubator at 150 rpm at 30° C for 3 – 7 days. After incubation the broth was aseptically collected in sterile centrifuge tubes and centrifuged at 2000 rpm for 20 minutes at 4°C in refrigerated centrifuge. The cell free supernatant (crude enzyme extract) was collected and enzymatic assay was performed in duplicates. 1 mL of 1% carboxy methyl cellulose (CMC) substrate was added to each 0.2 mL, 0.3 mL, 0.4 mL, 0.6 mL and 1 mL aliquots of enzyme solution; final volume upto 3 mL was made up with distilled water. Blank was prepared without addition of enzyme solution. The reaction mixture was incubated in waterbath at 60°C for 20 minutes. The reaction was stopped by addition of 1 mL DNS reagent and kept in boiling waterbath for 15 minutes. Final volume upto 10 mL was made up with distilled water after mixture is cooled to room temperature. Absorbance was taken at 540 nm. Standard of glucose (1 mg/mL) was prepared using DNS method. 1 unit (IU) is defined as the amount of enzyme that released 1µmole of glucose from carboxymethyl cellulose per minute at pH 7.0 at 60°C.

3) *Primary Screening for L- asparaginase Production*: The *Actinomyces* strains isolated were inoculated on L-asparagine glucose agar media and incubated at 30°C in an incubator for 3– 4 days. Pink zones around the L- asparaginase producing colonies were observed.

3.1) *L-asparaginase Assay*: The L-asparaginase producing positive strain obtained through primary screening was inoculated in 50 mL of L- asparagine glucose broth media and incubated in orbital shaking incubator at 150 rpm at 30° C for 3 – 7 days. After incubation the broth was aseptically collected in sterile centrifuge tubes and centrifuged at 10,000 rpm for 15 minutes at 4° C in refrigerated centrifuge. The cell free supernatant (crude enzyme extract) was collected and enzymatic assay was performed in duplicates. 0.5 mL of 1% L- asparagine substrate was added to each 0.1 mL, 0.3 mL, 0.5 mL, 0.7 mL and 1 mL aliquots of enzyme solution, to this 0.5 mL 0.05 M Tris HCl buffer (pH 7.2) was added and incubated at 37° C waterbath for 10 minutes. Reaction was stopped by addition of 0.5 mL 1.5 M Trichloroacetic acid (TCA). Then 0.2 mL of Nessler's reagent was added to each tube to detect the presence of ammonia released. Absorbance at 480 nm was taken after 2 minutes. Blank consist of distilled water and Nessler's reagent. Ammonium sulphate (1mM) was used as standard. One unit of asparaginase is the amount of enzyme which catalyzed the formation of 1µmol ammonia per min at 37°C.

4) *Primary Screening for Protease Production:* The strains isolated were inoculated on Skim milk agar media and incubated at 30° C in an incubator for 3 – 4 days. Distinct clear zone appears around the protease producing colonies.

4.1) *Protease Assay:* The protease producing positive strain obtained through primary screening was inoculated in 50 mL of skim milk broth media and incubated in orbital shaking incubator at 150 rpm at 30°C for 3 – 7 days. After incubation the broth was aseptically filtered through sterile Whatman filter paper no. 1, the filtrate obtained was used as crude enzyme. The enzymatic assay was performed in duplicates. 1 mL of 2% casein substrate was added to each 0.1 mL, 0.3 mL, 0.5 mL, 0.7 mL and 1 mL aliquots of enzyme solution, the reaction mixture was incubated at 37° C waterbath for 10 minutes. Reaction was stopped by addition of 5 % Trichloroacetic acid (TCA). The mixture was incubated at 37° C waterbath for 30 minutes. The mixture was filtered through Whatman filter paper no. 50, collect 2 mL of filtrate. To this 5 mL of 0.44 M sodium carbonate buffer and 1 mL diluted (1:2) Folin Ciocalteu (FC) reagent was added. The mixture was incubated at 37° C waterbath for 30 minutes. Blank consist of distilled water and FC reagent. Tyrosine (1 mg/mL) was used as standard. The amount of tyrosine released into the filtrate was measured at 660 nm absorbance. One unit of protease activity is expressed as the amount of enzyme which converts 1 µg of tyrosine per min at 37°C.

E. Screening for Antimicrobial and Antifungal Activity

The isolated *Actinomycetes* strains were screened for both antifungal and antibacterial activities. The isolates were inoculated in Nutrient broth media and incubated in orbital shaking incubator at 200 rpm for 3 – 7 days at 30° C. After incubation the inoculated broth was aseptically collected in sterile centrifuge tubes and centrifuged at 10,000 rpm for 20 minutes at 4° C in refrigerated centrifuge. Supernatant was filtered through Whatman filter paper no. 1, and filtrate was collected. The fungal test organisms include *Rhizopus*, *Aspergillus niger*, *Penicillium*, *Fusarium oxysporum* and *Neurospora crassa* were aseptically inoculated using sterile cotton swab on Potato dextrose agar plates. The bacterial test organisms include *Staphylococcus haemolyticus*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Serratia marcescens* were aseptically inoculated using sterile cotton swab on Mueller Hinton agar plates. Using sterile cork borer, bores were formed in each plate inoculated with test fungus and bacteria. Then 100 µL of filtrate from each isolate was loaded into the respective wells. Fungus inoculated plates were incubated at 30° C in an incubator in upright position for 24 hours, while those inoculated with bacteria were incubated at 37° C in upright position in an incubator for 24 hours. Zone of inhibition were observed and zone diameter was measured.

III. RESULTS

F. Isolation of Actinomycetes from Mangrove Soil

Total three *Actinomycetes* strains (two from sun and one from air dried) mangrove soil samples named as 1, 2 and 3 were isolated by both dilution-plate and direct streak methods. The growth was observed on all media used in the study except Potato dextrose agar media which did not support growth of any *Actinomycetes* from this soil sample. For further study all three strains were subcultured on Nutrient agar plates to obtain pure cultures and were stored at 4° C for further use.

G. Identification of Actinomycete Isolates

Colony characteristics of isolated strains are as shown in Table I. No growth of any strain was observed at pH 5.0, all strains grew at pH 7.0 and 9.0. All strains showed growth at 30°C and 40°C temperatures, while at 50°C no growth was observed. All strains showed growth at 5% NaCl. No growth was observed at 10% and 15% NaCl concentrations. All *Actinomycetes* strains 1, 2, and 3 were found to be positive for starch hydrolysis test. Strain 1 ferments all sugars except Rhamnose and Inositol sugars. Strain 2 does not ferment any sugar. Strain 3 is only able to ferment Cellulose and Xylose sugars. No strain produced gas. Fig.3 shows growth of strains at different pH, temperatures and NaCl concentrations.

TABLE I
MORPHOLOGICAL CHARACTERISTICS OF STRAINS ISOLATED

Morphological characteristics	Strain 1	Strain 2	Strain 3
Size	Medium	Small	Small
Shape	Round	Round	Round
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Flat
Opacity	Opaque	Opaque	Opaque
Consistency	Dry	Dry	Dry
Texture	Smooth	Smooth	Smooth
Odour	Camphor like/earthy	Earthy	Camphor like/earthy
Aerial mass colour	Whitish grey	Yellowish white	Reddish purple

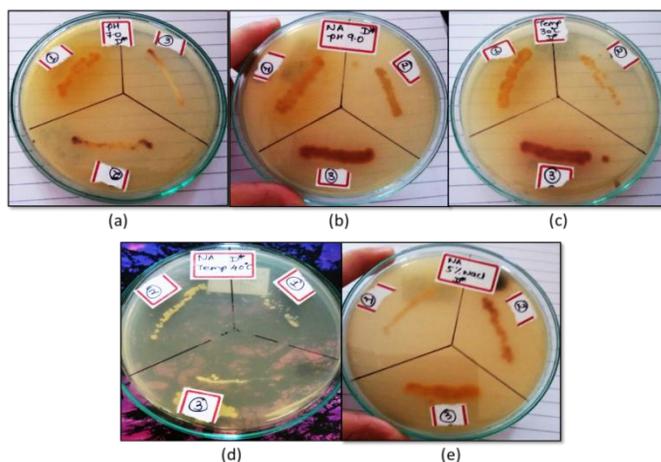


Fig. 3: Growth of all strains 1, 2, and 3 at (a) pH 7.0 (b) pH 9.0 (c) Temperature 30°C (d) Temperature 40°C (e) 5% NaCl concentration.

H. Enzyme Production and Enzyme Activity of Actinomycetes Strains Isolated

5) **Amylase Production:** Starch agar media plates inoculated with three strains were incubated for 3 days at 30°C was flooded with Gram's iodine solution. Clear distinct zone was produced around all three strains 1, 2, and 3, hence all strains produced amylase enzyme as shown in Fig.4.

5.1) **Amylase Assay:** Positive strains were checked further for amylase activity. Amylase activity was checked after three days incubation. Amylases hydrolyse starch into simple sugars like Maltose. Maltose (1mg/mL) was used as standard. Amylase activity was determined using DNS method. Strain 1 gives enzyme activity of 0.356 $\mu\text{mol/mL/min}$. Strain 2 gives enzyme activity of 0.465 $\mu\text{mol/mL/min}$. Strain 3 gives enzyme activity of 0.448 $\mu\text{mol/mL/min}$ on day 3 at 37° C and pH 7.0.

6) **Cellulase Production:** Czepak mineral salt agar media plates inoculated with three strains, after 3 days incubation at 30°C, was flooded with 0.5% Congo red solution and destained using 1 M NaCl solution. Clear yellow zone was produced around strain 1 and 3, hence producing cellulase enzyme as shown in Fig. 4.

6.1) **Cellulase Assay:** Positive strains were checked further for Cellulase activity. Cellulase activity was checked after third and seventh day of incubation. Cellulases hydrolyse cellulose into simple sugars like glucose. Glucose (1mg/mL) was used as standard. Cellulase activity was determined using DNS method. Strain 1 gives enzyme activity of 0.04 $\mu\text{mol/mL/min}$ on day 3 and 0.0388 $\mu\text{mol/mL/min}$ on day 7 at 60° C and pH 7.0. Strain 3 gives enzyme activity of 0.079 $\mu\text{mol/mL/min}$ on day 3 and 0.035 $\mu\text{mol/mL/min}$ on day 7 at 60° C and pH 7.0.

7) **L-asparaginase Production:** L- asparagine glucose agar media plates inoculated with three strains were after 3 days incubation at 30° C in an incubator was checked for pink zone formation around the colonies. Pink zone was produced around strain 2 and 3, hence they produce L- asparaginase enzyme as shown in Fig. 4.

7.1) **L- asparaginase Assay:** Positive strains were further checked for L- asparaginase activity. The activity was checked after both third and seventh day of incubation. L- asparaginase reacts with asparagine in media and produce aspartic acid and ammonia, thus the amount of ammonia liberated is measured at 480 nm. Ammonium sulphate (1 mM) was used as standard. Strain 2 gives enzyme activity of 0.0092 $\mu\text{mol/mL/min}$ on day 3 and 0.03 $\mu\text{mol/mL/min}$ on day 7 at 37° C. Strain 3 gives enzyme activity of 0.0124 $\mu\text{mol/mL/min}$ on day 3 and 0.027 $\mu\text{mol/mL/min}$ on day 7 at 37° C.

8) **Protease Production:** Skim milk agar media plates inoculated with three strains were after 3 days incubation at 30°C in an incubator was checked for clear distinct zone formation around the colonies. Clear distinct zone was produced around only strain 3, hence it produces Protease enzyme as shown in Fig. 4.

8.1) **Protease Assay:** Positive strain was further checked for Protease activity. The activity was checked after both third and seventh day of incubation. Protease digest casein, the major protein found in milk, and liberates tyrosine amino acid along with other amino acids and peptide fragments. Thus the amount of Tyrosine liberated reacts with Folin Ciocalteu reagent to give blue coloured product, which was measured spectrophotometrically at 660 nm. Tyrosine (1 mg/mL) was used as standard. Strain 3 gives enzyme activity of 1.837 $\mu\text{mol/mL/min}$ on day 3 and 3.32 $\mu\text{mol/mL/min}$ on day 7 at 37°C.

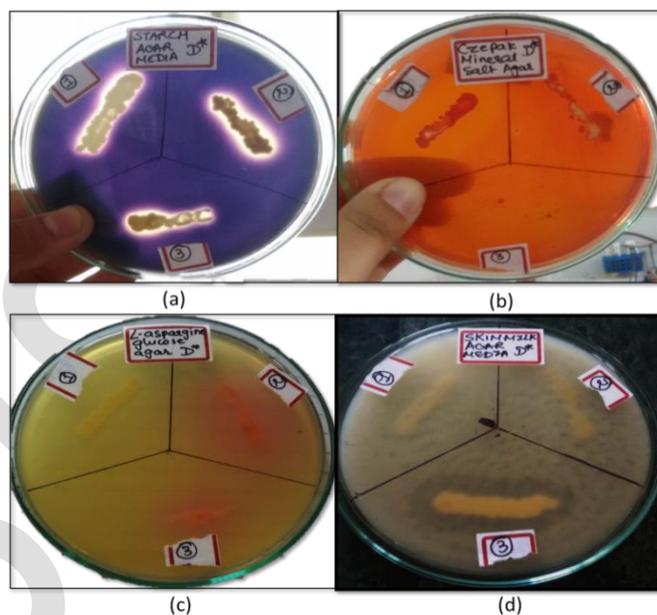


Fig. 4 Enzyme production (a) Amylase production by distinct clear zone formation around all strains 1, 2 and 3 isolated from mangrove soil, streaked on starch agar plate. (b) Cellulase production by distinct yellow zone formation around strains 1 and 3 isolated from mangrove soil, streaked on czepak mineral salt agar plate. (c) L- asparaginase production by pink zone formation around strains 2 and 3 isolated from mangrove soil, streaked on L- asparagine glucose agar plate. (d) Protease production by clear zone formation around strain 3 isolated from mangrove soil, streaked on skim milk agar plate

I. Antimicrobial and Antifungal Activity

Antimicrobial activity of all three strains was tested and among them strain 2 was found to have potential activity against *Salmonella typhimurium* with zone of inhibition of 8.5 mm shown in Fig. 5 (a) and *Bacillus cereus* with zone of inhibition of 15 mm shown in Fig. 5 (b) and strain 3 was found to have potential activity against *Serratia marcescens* with zone of inhibition of 10 mm shown in Fig. 5 (c). 7 days were found to be optimum incubation period for antimicrobial activity. No strain was found to have potential activity against *Staphylococcus haemolyticus*, and *Klebsiella pneumoniae*. Antifungal activity of all three strains was tested and no strain was found to have potential activity against test fungal organisms.

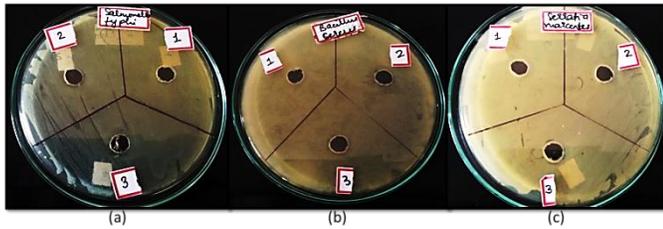


Fig. 5 Antimicrobial activity (a) Shows zone of inhibition of 8.5 mm by strain 2 against *Salmonella typhimurium*. (b) Shows zone of inhibition of 15 mm by strain 2 against *Bacillus cereus*. (c) Shows zone of inhibition of 10 mm by strain 3 against *Serratia marcescens*.

IV. DISCUSSION

Ismet *et al.* (2004) [6] and Hong *et al.* (2009) [7] reported isolation of *Actinomycetes* from mangrove soil. In this work soil sample was collected from Dumas – Bhimpore mangrove region and sample was sun dried and air dried. Goodfellow and Hynes (1984) reviewed the literature on isolation of actinomycetes from marine sediments and suggested that the marine sediment may be valuable for the isolation of novel actinomycetes. In this study, three *Actinomycetes* strains were isolated, two from sun dried soil sample and one from air dried soil sample. Thus sun dried soil sample was found better for *Actinomycetes* isolation. Holt *et al.* (1994) [8] identified the isolated actinomycetes based on the colony morphology and gram staining. The isolated strains were characterized by morphological, physiological and biochemical characteristics. The characterization by physiological and biochemical characteristics was done as described by Das *et al.* (2008) [3]. A study was done by Jeffrey *et al.* (2008) [9] by the collection of 62 isolates of actinomycetes isolated from soil samples collected from Agriculture Research Center Semongok, Sarawak. All the 62 isolates exhibit dark grey, grey, dark brown, brownish, whitish and yellowish white colors. We found the aerial mass colours of isolated strains as whitish grey, yellowish white and reddish purple. Kavya *et al.* (2012) [10] isolated few *Actinomycetes* strains that released characteristic camphor like smell. In present study two isolates were isolated that released camphor like earthy smell, whereas one isolate released only earthy smell. All strains were found to be Gram positive. Based on Shirling & Gottlieb (1966) [11] methods for *Streptomyces* spp. characterization, the basic observation should involve cultivation of cultures on various medium; yeast extract – malt extract agar (ISP2), oatmeal agar (ISP3), inorganic salts – starch agar (ISP4) and glycerol – asparagine agar (ISP5). Baskaran *et al.*, 2011 [1] utilized SCA media for isolation of *Actinomycetes*. Our results showed that all strains were able to grow on ISP-2, GAA, SCA, and NA media with no added antibiotics; they were unable to grow on PDA media. All isolates were able to grow at pH 7.0 and 9.0; with optimum growth at pH 9.0; this shows that they prefer alkaline environment and hence are alkaliphiles, they were able to grow at temperature 30°C and 40°C; with optimum growth at 40°C; thus are mesophiles, and shows growth at 5% NaCl concentration; thus can be kept in category of slight halophiles. All isolates were found to be positive for starch

hydrolysis test. Strain 1 ferments all sugars except rhamnose and inositol, while strain 2 was unable to ferment any sugar as there was colour change from red to dark pink and not yellow; hence such result may be due to strain producing nitrogenous products, strain 3 was able to ferment only cellulose and xylose sugars. Jeffrey *et al.* (2008) [9] isolated 48, 46 and 41 isolates that showed the ability to secrete the enzyme cellulase, lipase and protease respectively. We found in our study that all strains were able to produce amylase at 3% NaCl concentration. Strain 1 was found to be positive for amylase and cellulase production. Strain 2 was found to be positive for amylase and L-asparaginase production. Strain 3 amongst the other two strains is the only strain that is found to have potential to produce all four enzymes. Among all isolated strains, strain 2 was found to give more amylase activity (day 3) and L-asparaginase activity (day 7); strain 3 was found to give more cellulase activity (day 3) and L-asparaginase activity (day 3); strain 1 was found to give more cellulase activity (day 7). All the enzymes produced by isolated strains are of great industrial importance; such as amylase is used in food, textile, paper and detergent industries; cellulase is in great demand in pulp and paper industries, pharmaceutical applications and biofuel production; L-asparaginase is valuable for treatment of acute lymphoblastic leukemia and protease is useful in laundry detergents and bread industry. Baskaran *et al.*, 2011 [1] isolated 42 *Actinomycetes* strain. 22 strains showed antimicrobial potential against test bacteria namely, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumoniae*. Based on this study we also tested antimicrobial potential of the three isolates against five test bacteria namely; *Salmonella typhimurium*, *Bacillus cereus*, *Serratia marcescens*, *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*. Strain 2 was found to have potential antimicrobial activity against Gram negative *Salmonella typhimurium* and Gram positive *Bacillus cereus*. Strain 3 was found to have potential antimicrobial activity against Gram negative *Serratia marcescens*. Lim *et al.* (2000) [12] selected 32 *Actinomycetes* isolates, which showed the inhibitory activity against mycelial growth of plant pathogenic fungi like *Alternaria mali*, *Colletotrichum gloeosporoides*, *F.oxysporum*, *cucumerinum*, *Magnaporthe grisea*, *Phytophthora capsici*, and *Rhizoctonia solani*. We found no strain with potential antifungal activity against test fungal organisms such as *Rhizopus*, *Penicillium*, *Neurospora crassa*, *Aspergillus niger* and *Fusarium oxysporum*.

V. CONCLUSION

From the above study it can be concluded that mangrove ecosystem of Dumas region provides the rich source of enzyme producing and antimicrobial activity exhibiting *Actinomycetes* strains. Three *Actinomycetes* strains were isolated, of which strain 3 was found to produce all four enzymes and also showed antimicrobial activity. Strain 2 shows higher antimicrobial activity potential. Thus this isolates show great pharmaceutical and industrial potential.

More intensive study should be conducted on the isolated *Actinomycetes* to utilize potential *Actinomycetes* either as biocontrol or bioremediation agents. This isolates can also be further studied for more potent bioactive antibacterial and antifungal compound production against plant pathogenic bacteria and fungus.

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