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SK Chand Basha
Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

KRS Sambasiva Rao
(1) Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India
(2) Mizoram Central University, Aizawal, Mizoram, India

E Venkatanagaraju
Department of Life Sciences, CHRIST (Deemed to be University), Bangalore, Karnataka, India

FR Jobi Xavier
Department of Life Sciences, CHRIST (Deemed to be University), Bangalore, Karnataka, India

Correspondence
SK Chand Basha
Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India
E-mail: mahashaf@gmail.com

An evaluation of the geographical influence on the α-amylase bioactivity of actinomycetes - isolated from Coringa Mangroves

SK Chand Basha, KRS Sambasiva Rao, E Venkatanagaraju and FR Jobi Xavier

Abstract
Mangroves are the versatile and indomitable plant community for a wide range of bioactivities owing to its unique marine - terrestrial extreme interface. The objective of the current study is to evaluate the hypothesis, to what extent the geography (distance from the shore) influences the α-Amylase production from mangrovian actinomycetes. Six different soil samples (A_1, A_2, A_3, A_4, A_5 and A_6) were collected from Coringa mangroves based on shore to distant shore and were subjected to serial dilution and isolation techniques. A total of nine strains were identified based on colony characteristics. The primary screening for α Amylase recognized two strains (ANUKCJV1:132135 – A_5 and ANUKCJV2:130305 – A_2) as the potential candidate strains and were subjected to α-Amylase production, the two strains exhibited 0.491 and 0.303 IU of α-Amylase activity. The two potential strains were identified as *Streptomyces pluripotens* and *Streptomyces chilikensis* respectively based on 16S r DNA sequencing. The sequences of the two potential strains were deposited in the NCBI with accession codes; ANUKCJV1 – MH973320 and ANUKCJV2 – MH973321 respectively. Based on the current study it may be concluded that both the extremeness and favourability of the mangrovian environ influences the α-Amylase production.

Keywords: Mangroves, actinomycetes, α-amylase, *Streptomyces*

Introduction
Over 70% of planet earth is covered by marine strata and the origin of life begun from the same (Valli *et al*., 2012) [1]. Marine strata may serve as an indomitable hub for potential microbes. Biota is the ultimate source of all enzymes, owing to the enhanced proliferation rates of microbes; microbial based enzyme production may meet the growing market demand for various Industrial enzymes (Kumar and Takagi, 1999) [2]. The stable organic structure of microbial enzymes attracts commercial exploitation (Ramesh *et al*, 2009) [3]. Microbes occupying different strata of marine region enduring as potential sources of industrial enzymes (Selvam *et al*., 2011) [4]. Terrestrial actinomycetes were on decelerating trend there by prompting researchers to focus on diversified systems [1] in tune of it, marine regime found to be an attractive destination for bioactive exploration. Under the head of marine regime, mangrovian microbes in general, and actinomycetes in particular serves as the indomitable sources of industrial enzymes (Yu *et al*., 2015 [5]; Ramesh *et al*., 2009 [3]; Basha and Rao., 2017) [6]. Mangroves embodying 68 woody halophytic divergent plant groups possessing adaptations with respect to extreme transitional marine - terrestrial interface (Basha, 2018) [7]. The geographical extents of mangroves in the world are 30° S to 38° N (Mathew *et al*., 2010) [8]. Mangroves harbored in estuaries, lagoons, backwaters, sheltered shores, mudflats, creeks and marshes (MoEF 2014 – 15) [9]; (Sohangupta *et al*., 2015) [10]. Marine Actinomycetes in general and *Streptomyces* in particular yielded nearly half of the secondary metabolites (Basha and Rao., 2017) [6]; (Kathiresan and Manivannan, 2006) [11]. Despite this fact, still the bioactive exploration from mangroves actinomycetes is at infancy (Rajesh *et al*., 2011) [12]. Marine Actinomycetes are of diversified biotic origin embodying various ecological niches (Manivasagan *et al*., 2014) [13]; (Ward and Bora 2006) [14]. A novel actinomycetes *Nonomurea maheshkhaliensis* found to be a potential source of Menaquinone was isolated from mangroves (Ara *et al*., 2007) [15]. The DNA of Actinomycetes embodies high G – C (Guanine – Cytosine) content of over 55% which is unusual (Bonjar *et al*., 2005) [16]. The current study has been designed to evaluate the α-Amylase enzyme potentiality of under explored Coringa mangroves actinomycetes. Godavari delta which harbours Coringa mangroves, is one of the prominent delta of India's east coast which is an emergent type, its genesis is attributed to plate tectonics (Anonymous, n.d) [22]; (Ranadhir and Karisiddaiah, 2014) [23].

α Amylase (endo – 1- α – D – glucano hydrolases EC 3.2.1.1) which is a starch degrading enzyme found its applications in a wide variety of industries beginning with food, fermentation, paper to textile industries (Selvam *et al*., 2011) [4]. Researchers across the globe reported mangroves as the potential sources of α- amylase activity (Armando *et al*., 2009) [17]; (Sathya and Ushadevi, 2014) [18]; (Farshid and Faranak, 2015) [19]. Previously Naragani *et al* (2015) [21] conducted optimization studies for α Amylase enzyme production from *Streptomyces cheonanensis* VUK A which was isolated from Coringa mangroves, to the best of our knowledge it is the only report from the same. The current study was constructed based on the hypothesis that 'Impact of extreme transitional conditions of marine – terrestrial interface - unstable substratum, hyper salinity, daily tidal fluctuations and anaerobic conditions may be influenced by the geographical distance from the shore and in turn influences the industrial enzyme bioactivity of mangroves actinomycetes', accordingly six different soil samples (A_1 to A_6) were collected from Coringa mangroves by covering the entire ecosystem with considerable distance between and amongst different soil samples. This kind of geographical approach for α- amylase activity has not been tested and ours was the first in that line.

Materials and Methods

i. Sampling station: Core samples were collected from Coringa mangroves (N: 16º 41′ - 16º 53′ and E: 82º 14′ – 82º 21′) (Fig.1). The Coringa mangroves which has been designated as a sanctuary, is the second largest mangroves in India extending for 124 sq.km located in the east coast of India (Andhra Pradesh state) along the Kakinada – Godavari estuary, south of Kakinada Bay. Geographically Coringa is a sand pit and receives varied deltaic branches of Godavari and Gouthami rivers (Madhu, 2013 [24]; Satyanarayana *et al*. 2002) [25]. As discussed core sample collection was done vis – a- vis to distance from the shore i.e. totally 6 soil samples A_1, A_2, A_3, A_4, A_5 and A_6 were collected where-in, A_1 is the distant soil sample from the shore and A_6 is the shore soil sample. Considerable distance has been maintained between and amongst different soil samples, such that each and every soil sample is different from the other with respect to extreme environmental conditions – unstable substratum, tidal fluctuations, anaerobic conditions and hyper-salinity. Edaphic factors have also been considered during sampling. Samples were transferred to the laboratory in air tight bags for investigation.

Fig 1: Coringa Mangroves [36]

ii. Isolation of the Actinomycetes strains: The soil samples were air dried at room temperature, serial dilutions were performed (10^{-3} to 10^{-5}), spread plated on ISP 4 (International Streptomyces Project) media (Table 1) and kept for incubation at 26 ºC for 7 days with daily observation for actinomycetes growth, after incubation period, colonies were selected based on chalky leathery appearance and were purified on the same media and kept for incubation at 26ºC for 7 days (Sherling and Gotlib, 1966) [26].

Table 1: Composition of ISP 4 (inorganic Salt) media

Composition	g/ L
Starch, soluble	10.000
Dipotassium phosphate	1.000
Magnesiumsulphate. Heptahydrate	1.000
Sodium chloride	1.000
Ammonium sulphate	2.000
Calcium carbonate	2.000
Ferrous sulphate, Heptahydrate	0.001
Manganous chloride, 7H2O	0.001
Zinc sulphate, 7H2O	0.001
Agar	20

iii. Primary screening: Primary screening for α Amylase was performed by inoculating the strains on Agar media followed by flooding with Gram's Iodine, leaving it for 5 minutes, amylase producing strains were identified by the formation of the zone of clearance against the blue colour background (Naragani *et al*., 2015) [21].

iv. Secondary Screening for α Amylase / quantification of α Amylase: Secondary screening for α Amylase of potential strains was done as per the (Bernfield, 1995) [27] in production media (Table 2) reaction mixture consists of 0.5 ml of substrate solution and 0.5 ml of enzyme extract and subjecting it to 37ºC for 5 minutes. Dinitrosalicylate method (DNS method) was used to determine reducing sugars levels (Naragani *et al*., 2015) [21]. The reaction was terminated by the addition of 1 ml DNSA, Spectrophotometric analysis at 540 nm was done, and values were expressed in units (IU).

Table 2: The composition of Amylase Production Media

Composition	g/L
Na_2HPO_4	6 g
KH_2PO_4	3 g
NH_4Cl	1 g
NaCl	0.5g
$CaCl_2$	0.15
$MgSO_4$. $7H_2O$	0.25 g
Casein hydrolysate	0.20 g
Yeast extract	0.10 g
Starch	20 g

Molecular identification of the actinomycetes strains

Molecular approach has been followed for identification of the strains (Sivakumar, n.d.) [28]. DNA of the strains was evaluated and its quality was ascertained on 1.0% Agarose gel. The obtained 16S rDNA gene sequence was utilized for BLAST analyses with NCBI GeneBank data base and aligned using Clustal W (multiple alignment software programmes). MEGA 7 version was used for phylogenetic tree construction and Distance matrix generation (Kimura, 1980 [29]; Kumar *et al*., 2015 [30]; Felsenstein, 1985) [31].

Results

Isolation of the strains: 9 morphologically distinct colonies were isolated from 6 different soil samples (A_1, A_2, A_3, A_4, A_5 and A_6) surprisingly just one colony was isolated from A6 sample. The spore mass growth for the colonies (A_1, A_2, A_3

and A_5) exhibited ranges from Abundant (Fig. 2a), moderate to less growth whereas, for A_6 colony the spore mass was poorly grown. The pigmentation for aerial mycelium for obtained colonies ranges from white – grey (Fig. 2c) – yellow to light brown (Fig 2b). The pigmentation for reverse mycelium ranges from white – grey – light yellow – yellow – light brown to orange brown (Table.3).

Table 3: Colony characteristics of isolated Strains from Each soil Samples on ISP 4 Media.

Samples	No. of colonies	Appearance				
		Sl No	Spore mass	Ariel Mycelium	Reverse Mycelium	Pigmentation
A1	3	A1-142838	Abundant	White	Light Yellow	-
		A1-130236	Abundant	Grey	Grey	-
		A1-143301	Abundant	Grey	Yellow	-
A2	2	A2-130305	Moderate	Light brown	Light Brown	+
		A2-130352	-	Yellow	White	+
A3	1	A3-1510118	Less	White	White	-
A4	1	A4-Ye	-	Yellow	Yellow	-
A5	1	A5-132135	Abundant	Grey	Orange to Brown	+
A6	1	A6-131044	Poor Growth			

Primary Screening of the strains: All the morphologically distinct colonies were allowed to grow on ISP 4 media and kept for incubation at 26ºC for 7 days. After that the plates were flooded with grams Iodine, and allowed for zone of clearance formation, which exhibited that 5 strains were showing zone of formation (Fig. 3), out of which three strains (A5-132135, A2- 130305 & A2-130352) subjected to secondary screening / production based on potentiality.

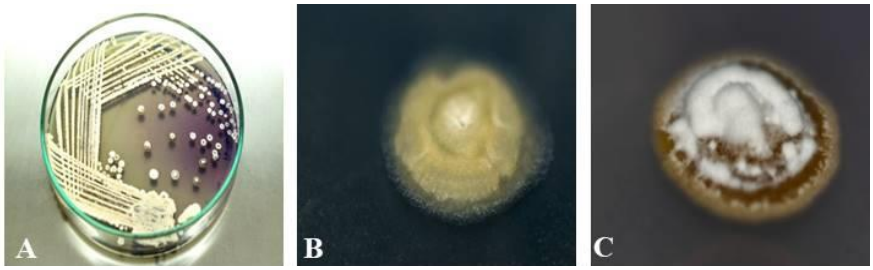

Fig 2: Actinomycetes colonies on ISP 4 media; A – Strain A_5-132135 Streak on ISP 4 Media; B - Strain A_2- 130305 Single Colony on ISP 4 Media; C - Strain A_5-132135 Single Colony on ISP 4 Media.

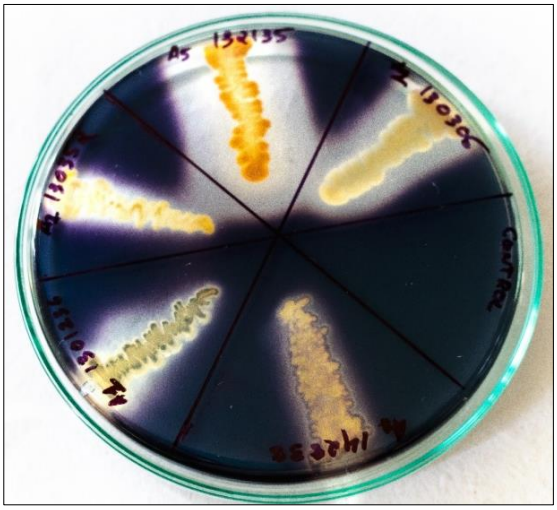

Fig 3: Starch hydrolysis Capability of selected Strains (A_5-132135 & A_2- 130305 showing more zone of clearance)

Secondary Screening of the strains: The three potential strains from primary Screening (A_5 - 132135, A_2 - 130305 and A_2 - 130352) were subjected to secondary screening / production through DNSA method. The volume of substrate taken was 0.5 ml and the crude enzyme fraction - 0.5 ml, the reaction was allowed at 37ºC for 5 minutes and absorption was measured at 540 nm for measuring its reducing sugar activity. The three strains A_5-132135; A_2-130305 and A_2-130352 exhibited the α-Amylase enzyme activity of 0.491, 0.303 and 0.064 IU respectively (Table 5). Maltose as standard was measured with different concentrations and volumes of distilled water, its respective OD values were measured at 540 nm where-in, at a lower con. of 200 and 400 µg/mL enzyme activity of 0.05 and 0.102 IU was exhibited and at higher concentrations of 1000 µg/mL 0.25 IU of enzyme activity was exhibited (Table 6). Maltose O.D. values were plotted in the form of a standard curve, it projects as a linear axis which suggest a constant rise in enzymatic activity with increase in maltose concentration, its R^2 value is taken as 0.9997 and y value as 0.0002x + 0.0004. (Chart. 1).

Secondary Screening results showed that two strains 132135 (A_5) and 130305 (A_2) were the potential strains and indicated as 132135 – ANUKCJV1 and 130305 – ANUKCJV2.

Table 4: Maltose standard Curve table

S. No.	Vol. Of Std (mL)	Concentration of Std (µg/mL)	Vol Of Distilled Water (mL)	Vol of DNSA (mL)	Incubation for 10 Mins in boiling water bath	Od Values (540 nm)
B	-	-	3	1		0
S1	1	200	4			0.05
S2	2	400	3			0.102
S3	3	600	2			0.148
S4	4	800	1			0.197
S5	5	1000	0			0.25

Table 5: Amylase Activity table

S. No	Volume of the substrate (mL)	37ºC for 5 mins	Volume of Enzyme (mL)	37ºC For 5 mins	Volume of DNSA (mL)	Boiling water bath for 5 mins	OD value (540nm)
A5-132135	0.5		0.5		1		0.491
A2-130305							0.303
A2-130352							0.064

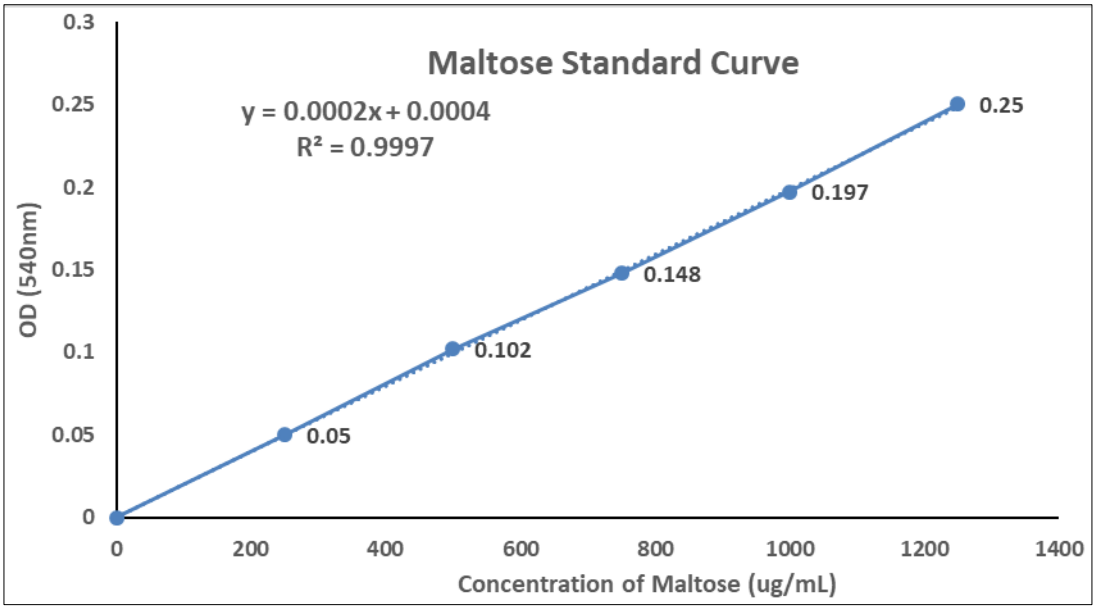

Chart 1: showing the standard curve for Maltose

Molecular identification of the potential strains: Initially the integrity and quality of DNA for the two strains 132135 (A_5) (ANUKCJV1) and 130305 (A_2) (ANUKCJV2) has observed by agarose gel electrophoresis. This high quality DNA was used for PCR amplification of 16S rDNA gene fragment by using primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492 R (5' TACGGYTACCTTGTTACGACTT 3'). As expected a 1500 bp PCR amplicon was observed for both the strains. The PCR amplicon of respective strains was further subjected to forward and reverse DNA sequencing generating a consensus sequence of 16S rDNA gene. The obtained 16S rDNA gene sequence was subjected to BLAST with data base inputs from NCBI GenBank. The Clustal W multiple alignment software programmes was used for alignment of top ten sequences which were generated based on score of maximum identity, where *Streptomyces pluripotens* strain MUSC 135 16S ribosomal RNA gene, partial sequence for ANUKCJV1 - 132135 (A_5) (Table. 6) and *Streptomyces chilikensis* strain RC 1830 16S ribosomal RNA gene, partial sequence for ANUKCJV2 - 130305 (A_2) (Table.7) found to exhibit much similarity when compared to rest of the nine closely related sequences

The generated bootstrap consensus tree which was inferred from 1000 replicates was used for analyses of the evolutionary history of the taxa. Initially the phylogenic tree was constructed by applying the Neighbor - Join and BioNJ algorithms followed by the topology selection based on superior log likelihood value. The algorithmic analyses involved eleven nucleotide sequences with codon positions comprising of 1st, 2nd, 3rd and non coding. A total of 1434 positions were generated in the final dataset, finally phylogenetic tree was established based on MEGA 7 software analyses for both the strain 132135 and 130305 (Fig. 4 and 5). An estimate of evolutionary divergence between Sequences was done leading to the generation of Distance matrix for the two strains 132135 and 130305 (Tables 8 & 9) which documented the number of base substitutions. Kimura two parameter model was conducted for analyses which involved 11 nucleotide sequences with positions of codons 1st, 2nd, 3rd and non coding and finally evolutionary analyses was done based on MEGA 7 software. The sequences of the two potential strains were deposited in the NCBI with accession codes; ANUKCJV1 – MH973320 and ANUKCJV2 – MH973321 respectively.

Table 6: Sequences producing significant alignments for the strain 132135

Description	Max score	Total score	Query cover	E value	Ident	Accession
Streptomyces pluripotens strain MU5C 135 16S ribosomal RNA gene, partial sequence	1764	1764	100%	0	99%	NR_126290.1
Streptomyces chiangmaiensis strain TA4-116S ribosomal RNA gene, partial sequence	1759	1759	100%	0	99%	NR_113180.1
Streptomyces mexicanus strain NBRC 100915 16S ribosomal RNA gene, partial sequence	1759	1759	100%	0	99%	NR_041429.1
Streptomyces mexicanus strain CH-M-1 035 16S ribosomal RN Agene, partialsequence	1759	1759	100%	0	99%	NR_114647.1
Streptomyces chromofuscus strain NBRC1 2851 16S ribosomal RNA gene, partialsequence	1753	1753	100%	0	99%	NR_041082.1
Streptomyces leeuwenhoekii strain C341 6S ribosomal RNA gene, partial sequence	1748	1748	100%	0	99%	NR_126200.1
Streptomyces bullii strain C21 6S ribosomal RNA gene, partial sequence	1748	1748	100%	0	99%	NR_108868.1
Streptomyces cinereospinus strain NBRC15397 16S ribosomal RNA gene, partialsequence	1748	1748	100%	0	99%	NR_041174.1
Streptomyces thermoviolaceus strain NBRC13905 16S ribosomal RNA gene, partialsequence	1748	1748	100%	0	99%	NR_112423.1
Streptomyces cinereospinus strain C55P426 16S ribosomal RNAgene, partial sequence	1748	1748	100%	0	99%	NR_115378.1

Table 7: Sequences producing significant alignments for the strain 130305

Description	Max score	Total score	Query cover	E value	Ident	Accession
Streptomyces chilikensis strain RC1830 16S ribosomal RNAgene, partial sequence	1600	1600	100%	0	99%	NR_118246.1
Streptomyces variabilis strain NRRLB-3984 16S ribosomal RNA gene,partial sequence	1600	1600	100%	0	99%	NR_043840.1
Streptomyces gougerotii strain NBRC13043 16S ribosomal RNAgene,partial sequence	1600	1600	100%	0	99%	NR_112610.1
Streptomyces rutgersensis strain NBRC 3727 16S ribosomal RNA gene,partial sequence	1600	1600	100%	0	99%	NR_112495.1
Streptomyces rutgersensis strain NBRC 3419 16S ribosomal RNA gene,partial sequence	1600	1600	100%	0	99%	NR_112488.1
Streptomyces labedae strain NBRC 15864 16S ribosomal RNA gene, partial sequence	1600	1600	100%	0	99%	NR_041192.1
Streptomyces erythrogriseus strain NBRC 1460116S ribosomal RNA gene, partial sequence	1600	1600	100%	0	99%	NR_112438.1
Streptomyces diastaticus strain NBRC1341216S ribosomal RNAgene,partial sequence	1600	1600	100%	0	99%	NR_112395.1
Streptomyces matensis strain NBRC1288916Sribosomal RNA gene, partial sequence	1600	1600	100%	0	99%	NR_041088.1
Streptomyces fragils strain NBRC 12862 16S ribosomal RNA gene, partial sequence	1600	1600	100%	0	99%	NR_112306.1

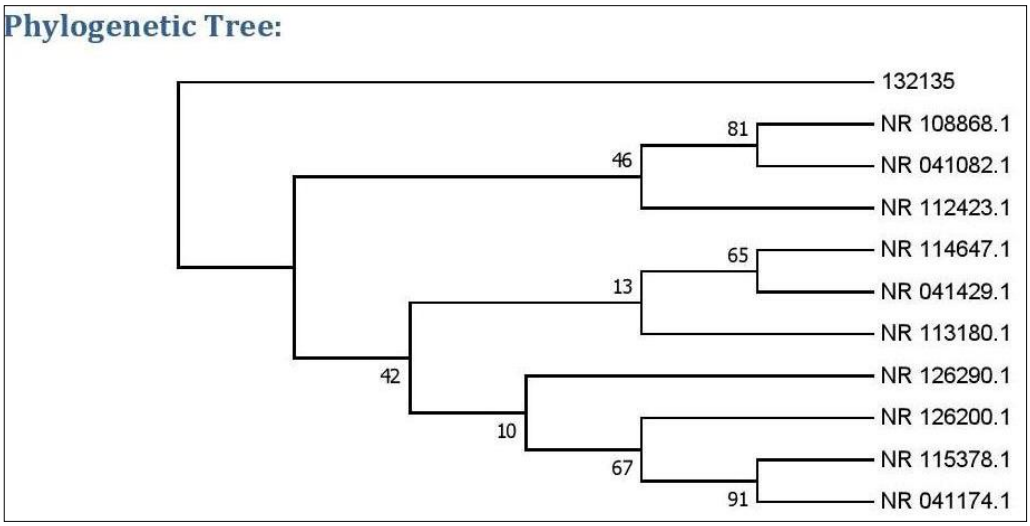

Fig 4: Phylogenetic tree for the strain 132135

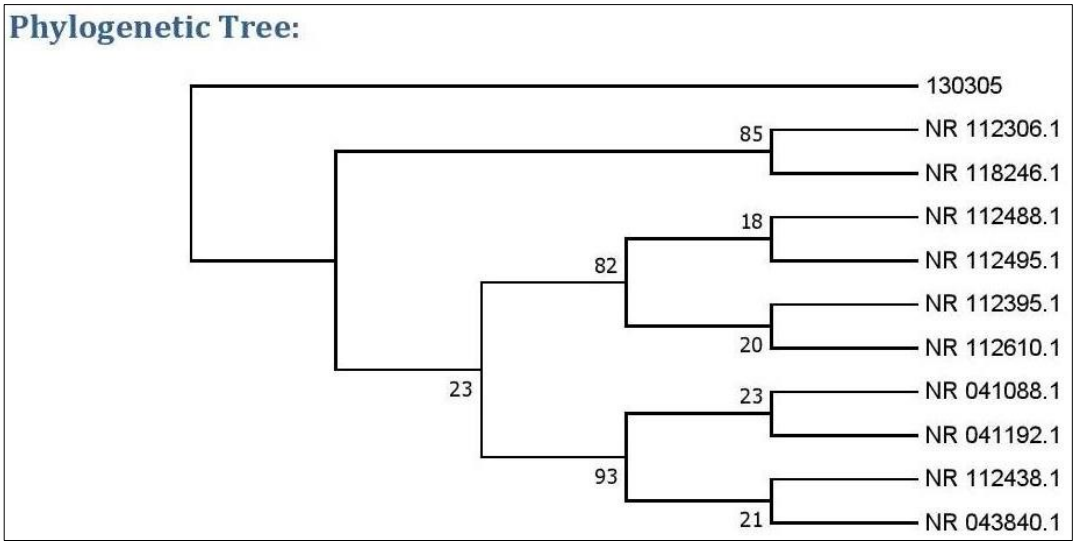

Fig 5: Phylogenetic tree for the strain 130305

Table 8: Data matrix for the strain 132135

132135		0.0010	0.0015	0.0014	0.0014	0.0017	0.0020	0.0021	0.0021	0.0020	0.0021
NR 126290.1	0.0010		0.0011	0.0009	0.0009	0.0014	0.0018	0.0018	0.0018	0.0023	0.0018
NR 113180.1	0.0021	0.0010		0.0014	0.0014	0.0018	0.0020	0.0021	0.0021	0.0026	0.0021
NR 041429.1	0.0021	0.0010	0.0021		0.0000	0.0017	0.0020	0.0020	0.0020	0.0025	0.0020
NR 114647.1	0.0021	0.0010	0.0021	0.0000		0.0017	0.0020	0.0020	0.0020	0.0025	0.0020
NR 041082.1	0.0031	0.0021	0.0031	0.0031	0.0031		0.0022	0.0011	0.0022	0.0023	0.0022
NR 126200.1	0.0042	0.0031	0.0042	0.0042	0.0042	0.0052		0.0024	0.0019	0.0028	0.0019
NR 108868.1	0.0042	0.0031	0.0042	0.0042	0.0042	0.0010	0.0063		0.0025	0.0025	0.0025
NR 041174.1	0.0042	0.0031	0.0042	0.0042	0.0042	0.0052	0.0042	0.0063		0.0030	0.0000
NR 112423.1	0.0042	0.0052	0.0063	0.0063	0.0063	0.0052	0.0084	0.0063	0.0084		0.0030
NR 115378.1	0.0042	0.0031	0.0042	0.0042	0.0042	0.0052	0.0042	0.0063	0.0000	0.0084	

Table 9: Data matrix for the strain 130305

130305		0.0024	0.0025	0.0024	0.0024	0.0024	0.0025	0.0025	0.0024	0.0025	0.0024
NR 118246.1	0.0057		0.0022	0.0016	0.0016	0.0016	0.0022	0.0022	0.0016	0.0022	0.0000
NR 043840.1	0.0057	0.0046		0.0022	0.0022	0.0022	0.0000	0.0000	0.0022	0.0000	0.0022
NR 112610.1	0.0057	0.0023	0.0046		0.0000	0.0000	0.0022	0.0022	0.0000	0.0022	0.0016
NR 112495.1	0.0057	0.0023	0.0046	0.0000		0.0000	0.0022	0.0022	0.0000	0.0022	0.0016
NR 112488.1	0.0057	0.0023	0.0046	0.0000	0.0000		0.0022	0.0022	0.0000	0.0022	0.0016
NR 041192.1	0.0057	0.0046	0.0000	0.0046	0.0046	0.0046		0.0000	0.0022	0.0000	0.0022
NR 112438.1	0.0057	0.0046	0.0000	0.0046	0.0046	0.0046	0.0000		0.0022	0.0000	0.0022
NR 112395.1	0.0057	0.0023	0.0046	0.0000	0.0000	0.0000	0.0046	0.0046		0.0022	0.0016
NR 041088.1	0.0057	0.0046	0.0000	0.0046	0.0046	0.0046	0.0000	0.0000	0.0046		0.0022
NR 112306.1	0.0057	0.0000	0.0046	0.0023	0.0023	0.0023	0.0046	0.0046	0.0023	0.0046	

Discussion

Since late 1980's production of novel compounds from terrestrial actinomycetes were on a declining trend, which prompted researchers to explore the marine region (Ramesh *et al*, 2009) [3]. Varied reports showed that actinomycetes are the versatile sources for unique secondary metabolites, particularly antibiotics and industrial enzymes viz. cellulase, amylase, lipase, gelatinase and chitinase (Bredholt *et al*., 2008) [32]. The Mangrove actinomycetes serves as the potential bioactive hub for the α - Amylase enzyme, the present results were in general agreement with the available reports. The experiment was designed to test the hypothesis that 'Impact of extreme transitional conditions of marine – terrestrial interface - unstable substratum, hyper-salinity, daily tidal fluctuations and anaerobic conditions may be influenced by the geographical distance from the shore and in turn influences the industrial enzyme bioactivity of mangroves actinomycetes' accordingly, 6 different soil samples were collected A_1, A_2, A_3, A_4, A_5 and A_6 respectively (A_1 is the distant and A_6 is the shore sample). Our study was the first to report α – Amylase production coming under geographical impact (distance from the shore). Surprisingly, A_6 soil sample

which is the shore sample is devoid of any potential strain, which we presume that it may be the potential source for α – Amylase, interestingly A_2 soil sample reported one of the potential strain A_2 – 130305 (ANUKCJV2) and its identification based on 16S rDNA sequencing as *Streptomyces chilikensis*, where-as A_5 soil sample reported the potential strain A_5 – 132135 (ANUKCJV1) and its identification based on 16S rDNA sequencing as *Streptomyces pluripotens*. Totally 9 actinomycetes strains were isolated from 6 different soil samples (A_1 to A_6), 3 A_1 samples (A_1 – 142838, A_1 – 130236 and A_1 – 143301), 1 A_3 sample (A_3 – 1510118), 1 A_4 sample (A_4- Ye), 1 A_5 sample (A_5 – 132135) and 1 A_6 sample (A_6 – 131044). Isolated strains depicts that marine sediments are the predominant sources of actinomycetes, previously Meena *et al*., 2013 [20] reported 26 actinomycetes strains from Andaman and Nicobar island's marine sediments, whereas Farshid and Faranak, 2015 [19] reported 86 strains of actinomycetes in spring and 61 actinomycetes strains in summer from mangroves of Nayband gulf of Southern Iran, which showed that mangroves are the hub for marine actinomycetes, we presume that the reason for greatest isolation of number of actinomycetes strains (86) from mangroves of southern Iran is may be due to favourable climatic factors. Our results states that out of 9 actinomycetes strains 5 strains exhibited starch hydrolysis i.e. over 50% of the strains reported α – Amylase bioactivity, in terms of quantum of potential actinomycetes strains our results were better than Selvam *et al*., 2011 [4] who reported 3 actinomycetes strains out of 56 strains which exhibited α – Amylase bioactivity which tantamount to around 5%.

As discussed two potential strains (A_5; 132135 – ANUKCJV1 and A_2; 130305 – ANUKCJV2) out of 5, reported as sources for α – Amylase activity and no bioactivity is reported from A6 shore soil sample, which contradicts our hypothesis. We presume that may be the favourability of environmental conditions like pH and the temperature may influence the bioactivity rather than wholly the extremeness. The secondary screening of two potential strains reported quite good promising results with A_5 – 132135 reported 0.491 U/ml and A_2 – 130305 reported 0.303 U/ml. Previous reports showed that marine Actinomycetes were the potential sources of α – Amylase activity. Selvam *et al*., 2011 [4] reported 6.48 U/ml of α – Amylase production from marine Actinomycetes, isolated from different locations of southern Indian coast – Chennai, Tuticorin, Kerala and Poombukar. Similarly, Meena *et al*., 2013 [20] reported *Streptomyces sp*. NIOT – VKKMA02 isolated from Andaman and Nicobar islands with 13.27 U/ml of α – Amylase production. Previously Naragani *et al*., 2015 [21] conducted optimization studies on Streptomyces cheonanensis VUK – A isolated from Coringa mangroves and reported α – Amylase production of 11.2 U/ml under peptone as nitrogen source, the essential distinguishing note with our findings was their findings were based on bulk analysis of Coringa mangrove soil whereas, our findings were based on geographical approach with different soil samples collected from shore to distant location from shore (A_6 to A_1).

Farshid and Faranak, 2015 [19] reported potential *Streptomyces* strains from mangroves of southern Iran with average α – Amylase production of 62.97 U/ml in the spring season, their findings were based on the seasonal impact on α – Amylase production. Satya and Ushadevi., 2018 [33] isolated *Streptomyces sp.* from Muthupet mangroves of Tamilnadu, India with α – Amylase activity in the range of 2.4± 0.002 – 5.9 ± 0.005 U/ml. Jaralla *et al*., 2014 [34] reported potential Streptomyces strains from Hillah region of Iraq with good α – Amylase activity. Similar to that Armando *et al*., 2009 reported different actinomycetes groups from *Cananeia sp*. mangroves of Brazil with good amylolytic activity. Sathya, and Ushadevi, 2014 [18] also reported potential α – Amylase *Streptomyces* strains from Muthupet mangroves of Tamilnadu, India. Different researchers also reported α – Amylase activity from varied microbes of mangroves and marine sources which were in line to our findings. Gurudeeban *et al*., 2011 [35] conducted optimization studies on *Bacillus megaterium* isolated from *Avicennia marina* (white mangroves) and reported α – Amylase activity as 156 U/ml under maltose (carbon source) and 140 U/ml under peptone (nitrogen source). Kathiresan and Manivannan., 2006 conducted optimization studies on *Pencillium fellutanum* isolated from *Rhizophora annamalayana* and reported α – Amylase production enhanced under peptone (nitrogen source) to 150 U/ml and Maltose (carbon source) to 146 U/ml.

Our findings reported two potential strains (A_5; 132135 – ANUKCJV1 and A_2; 130305 – ANUKCJV2) for α – Amylase production. The reported strains were subjected to 16S rDNA sequencing for Identification and reported A_2 – 130305 as *Streptomyces chilikensis* and A_5 – 132135 as *Streptomyces pluripotens*. Though our findings corroborate with the current findings, they contradict in terms of quantum of α – Amylase produced. Our results were in partial agreement with the proposed Hypothesis; in the way of the industrial enzyme bioactivity is influenced both by the extremeness and favourability of the environmental conditions. Ours was the first report to show how far the geography influences the α – Amylase activity of Coringa mangroves actinomycetes.

Conclusion

Coringa mangroves are one of the potential hub of industrial enzyme bioactivity, our results suggest this and in corroboration with the current findings. The geographical approach of the industrial enzyme bioactivity suggest that extreme environmental conditions do play a crucial role in α – Amylase production as the potential strain ANUKCJV1 – 132135 was isolated from A_5 shore sample, in addition to it, the favourability of the environ also influences the α – Amylase production in the way of ANUKCJV2 - 130305 which was isolated from A_2 shore sample. Though our findings were not holistically conclusive, it is the first step in that direction, further research required on the geographical approach of industrial enzyme bioactivity for expanding the knowledge basket.

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