Eco. Env. & Cons. 27 (February Suppl. Issue) : 2021; pp. (S48-S57)

ISSN 0971–765X

Comparative Optimization Studies (ISP 4 Vs ISP 3 Vs ISP 2 Media) of Mangrovian *Streptomyces pluripotens* ANUKCJV1 for its α - Amylase Production and Geographical Correlation of Mangrovian Actinomycetes strains

***SK. Chand Basha[1], K.R.S. Sambasiva Rao[2] and E. Venkatanagaraju [3]**

[1]Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur 522 510, A.P. India
[2]Mizoram Central University, Aizawal 796 004, Mizoram, India
[3]Department of Life Sciences, CHRIST (Deemed to be University), Bengalore 560 029, Karnataka, India

(Received 21 June, 2020; Accepted 5 August, 2020)

ABSTRACT

Streptomyces pluripotens ANUKCJV1 was isolated from Coringa Mangroves which was located along the South Indian Delta. The Current work which was in continuation to our previously reported work which suggests that *Streptomyces pluripotens* ANUKCJV1 was the potential strain and the same has been subjected to comparative optimization studies in the current work by employing three media: ISP 4; ISP 3; ISP 2 media for enhanced α - Amylase Production. Physico - Chemical variables viz… Incubation period, P^H, Temperature, Carbon and Nitrogen sources with respect to three different media (ISP 4, ISP 3 and ISP 2) were tested and cumulative analysis of three different media for differential bioactivity of α - Amylase was done. Results suggest that ISP 4 found to be the best medium with cumulative value of 24.2 U/mL, where as the cumulative value of ISP 3 and ISP 2 were 19.3 U/mL and 19.4 U/mL respectively. Peptone as Nitrogen source of ISP 4 found to be the favourite Individual variable among all with production value of 8.0 U/mL. Geographical correlation with respect to number of Actinomycetes strains and α - Amylase Bioactivity depicts that Distant geographical soil samples from the shore found to be favourable for higher number of Actinomycetes strains: A_1 soil samples (~ 500 m) - 33 %; A_2 samples (~ 400 m) - 22 %. With regard to α - Amylase Bioactivity, A_5 samples (~ 100 m) analysed to be the potential geographical bioactive zone for α - Amylase Production. From the study it can be concluded that since ISP 4 found to be the favourite medium of the potential strain, by employing the same large scale exploration of the *Streptomyces pluripotens* ANUKCJV1 of the Coringa Mangroves may be done to tap the industrial benefits of α - Amylase.

Key words: *Mangroves, Geography, α - Amylase and Streptomyces pluripotens ANUKCJV1*

Introduction

Microbes which belonged to different niches of marine ecosystem were the prudent and potential industrial enzyme hub (Selvam *et al*., 2011). Since the terrestrial ecosystem is unable to cope up with growing demand of novel bioactive compounds, search for novel ecosystem is a dire need (Valli *et al*.,

2012). Mangroves are one of the essential substrata of marine region, microbes in habituating the same; specifically Actinomycetes were the excellent sources of Industrial enzymes whose potentiality is second to none (Ramesh *et al*., 2009). The current work is in continuation to our previously reported work (Chand Basha *et al*., 2019) where in, two potential strains *Streptomyces pluripotens* ANUKCJV1 and *Streptomyces chilikensis* ANUKCJV2 were reported from Coringa mangroves under the lens of Geography.

The Best potential strain *Streptomyces pluripotens* ANUKCJV1 was subjected to comparative optimization studies. The potential strain which was isolated from A_5 Geographical soil sample (A_5 - 132135) was collected from ~ 100 m from the shore area in Coringa mangroves. Different workers reported optimization studies; Naragani *et al*. (2008) conducted optimization studies on *Streptomyces cheonanensis* VUK - A. Yassien and Asfour (2012) conducted optimization studies on *Streptomyces clavifer*. Mangamuri *et al.* (2011) tested Rare Actinomycetes strain *Pseudonocardia* sp. VUK - 10 based on comparative media study. Our work differs with the present knowledge in the way of testing three different media in optimization studies to decipher the differential - α Amylase bioactivity of the potential strain based on cumulative media analysis. Geographical correlation was done to assess to what extent the geography influences the α - Amylase production in mangrovian environment, in this instance Coringa Mangroves.

Materials and Methods

Optimization of Physico - Chemical Variables for α - Amylase enhanced production by the potential strain *Streptomyces Pluripotens* ANUKCJV1 by using ISP 4 Medium

(i) Impact of Incubation period on Production of α - Amylase: The Potential Actinomycetes strain's 1 week old culture was employed for suspension preparation which was inoculated into the broth of ISP 4, followed by fermentation for a period of 7 days at 30°C. Later, cultural filtrate was subjected to Analysis of α-Amylase and the measurement of biomass as dry weight (mg/mL) was done (Naragani *et al*., 2008).

(ii) Impact of pH on Production of α - Amylase: α **- Amylase** was subjected to ISP 4 broth inoculation with pH range of 5 – 30 °C incubation under shaking fermentation. After Incubation period of 72 hours α - Amylase production was measured. The optimum pH was used for further analysis.

(iii) Impact of Temperature on Production of α - Amylase: The Potential Actinomycetes strain was inoculated into the ISP 4 with temperature range of 25 °C – 45 °C at an inoculation period of 72 hours. The optimum temperature was employed for further study.

(iv) Influence of Carbon sources on the Production of α - Amylase: Different sources of Carbon - Sorbitol, Sorghum flour, Starch, Sucrose, Rice flour and others were added as supplements individually @ 10 mg/mL (w/v) to ISP 4 broth to assess the Influence of Carbon sources on production of α - Amylase.

(v) Impact of Nitrogen Sources on the Production of α - Amylase: Different Nitrogen sources : Beef extract, Peptone, Ammonium sulphate, Malt extract, Tryptone, Urea, Tyrosine, Yeast extract, Ammonium oxalate and Potassium nitrate individually @ 2 mg/mL were supplemented to ISP 4 broth to assess the Influence of Nitrogen.

ANOVA: Used for Statistical analysis of Biomass and Production of α-Amylase

Comparative media analysis (ISP 2 and ISP 3 media) based on cumulative data for construing of α - Amylase differential bioactive behaviour

Optimization of Physico - Chemical variables of *Streptomyces pluripotens* ANUKCJV1 (Potential Actinomycetes strain) was also done with ISP 2 and ISP 3 media besides ISP 4 medium. The objective of this work is to construe the α - Amylase differential bioactive behaviour based on cumulative data analysis of individual medium (ISP 4 Vs ISP 3 Vs ISP 2). The cumulative data of individual medium (ISP 4, ISP 3 & ISP 2) comprises of favourable variables: Incubation period, pH, Temperature, Carbon and Nitrogen Sources. Comparative media analysis was done pertaining to cumulative data of Individual medium (Mangamuri *et al*., 2011).

Geographical Correlation part (Analysis)

This part of the work comprises of overall geographical correlation of the primary work (The current paper is the extension of the same) in the way of its influence on the number of Actinomycetes strains and α - Amylase bioactivity. 6 different geo-

graphical soil samples were collected in Coringa mangroves of South Indian delta designated as A_1 soil sample - ~ 500 m away from the shore; A_2 soil sample - ~ 400 m away from the shore; A_3 soil sample - ~ 300 m away from the shore; A_4 soil sample - ~ 200 m away from the shore; A_5 soil sample - ~ 100 m away from the shore; A_6 soil sample - 0 m away from the shore. The objective is to analyse to what extent the geographical distance from the shore influences the number of strains of Actinomycetes and α - Amylase production. The analysis part was done with suitable figures and graphs.

Results

I. Optimization of experimental variables for α - Amylase enhanced production by *Streptomyces pluripotens* ANUKCJV1 (Potential Actinomycetes strain) by employing ISP 4 medium

(i) Influence of Incubation period on growth: Biomass of the potential Actinomycetes strains in ISP 4 broth reported progressive enhancement up to 72 hours period with 150 mg/100 ml as the highest biomass, later on the rate of biomass took deceleration trend (Fig. 1).

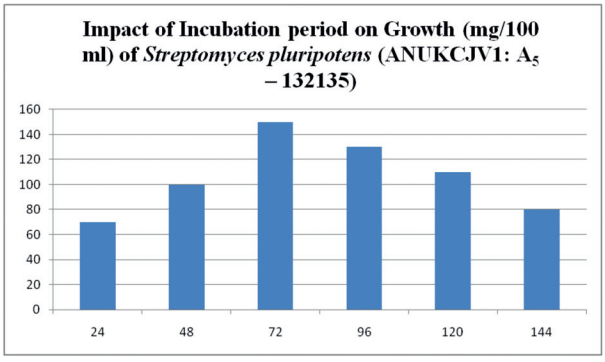


Fig. 1. Influence of Incubation period on growth

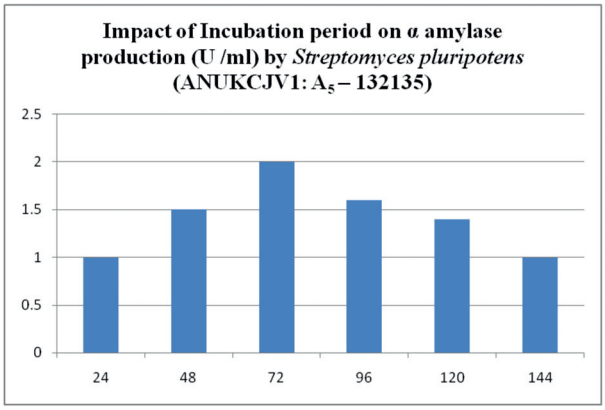


Fig. 2. Influence of Incubation period on production of α-Amylase

(ii) Influence of Incubation period on production of α - Amylase: The accelerating trend of α - Amylase enhanced production was reported by potential strain from 24 hours up to 72 hours and shows deceleration thereafter (Fig. 2).

(iii) Impact of pH on production of α - Amylase: Gradual rise in production of α - Amylase was reported from pH 5.0 - 7.0 with 4.0 U / mL reported as the highest production at pH 7.0, further the production of α - Amylase shows decelerating trend up to pH 9.0 (Fig. 3).

(iv) Influence of Temperature on production of α -Amylase: The Potential strain of Actinomycetes reported 4.2 U/mL as the highest α - Amylase yield at 35 °C and 1.0 U/mL as the lowest yield at 25 °C (Fig. 4).

(v) Influence of Carbon Sources on production of α - Amylase: Different Carbon Sources were in supplemented to the ISP 4 broth individually

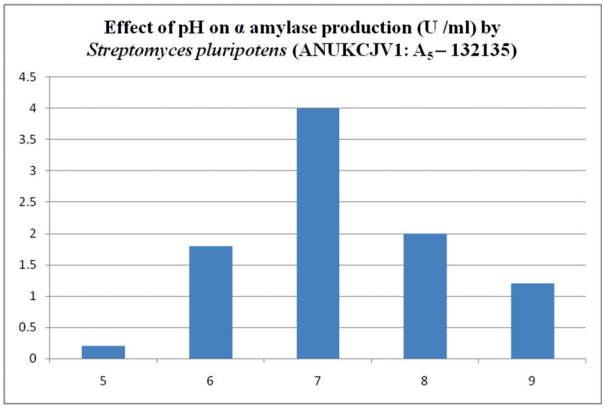


Fig. 3. Impact of pH on production of α - Amylase

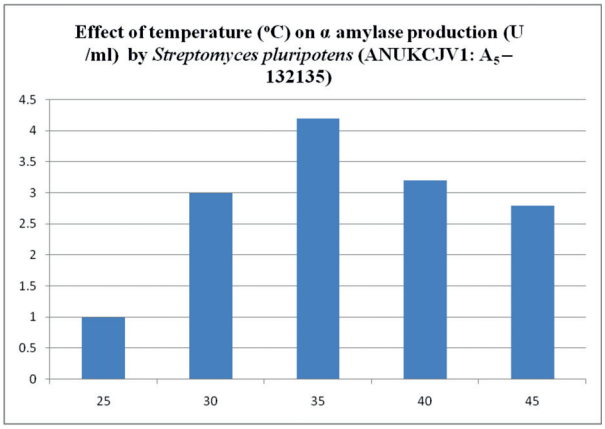


Fig. 4. Influence of Temperature on production of α - Amylase

where in, Sorghum flour with production of 6.0 U/mL reported as the best carbon source (Fig. 5).

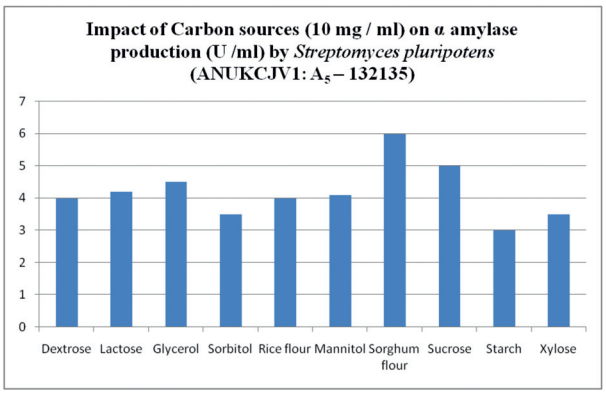


Fig. 5. Influence of Carbon Sources on production of α - Amylase

(vi) Influence of Nitrogen Sources on production of α - Amylase: Different Nitrogen Sources were supplemented to the ISP 4 broth individually, out of which Peptone with production value of 8.0 U / mL reported as the best Nitrogen source (Fig. 6).

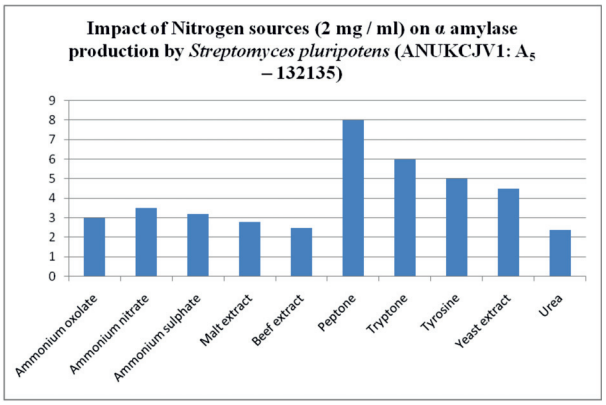


Fig. 6. Influence of Nitrogen Sources on production of α -Amylase

II. Optimization of experimental variables for α - Amylase enhanced production by *Streptomyces pluripotens* ANUKCJV1 (Potential Actinomycetes strain) by employing ISP 3 medium

(i) Influence of Incubation period on growth: Highest growth of Biomass was reported at 72 hours (Fig. 7).

(ii) Influence of Incubation period on production of α - Amylase: At 72 hours of Incubation period highest yield of α - Amylase was produced (Fig. 8).

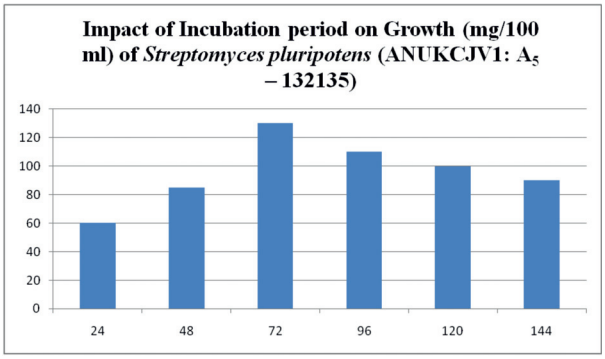


Fig. 7. Influence of Incubation period on growth

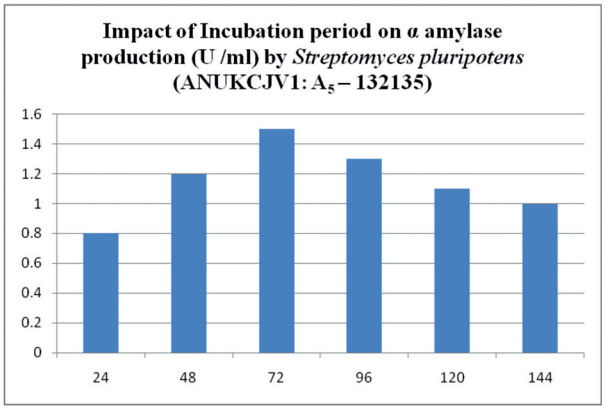


Fig. 8. Influence of Incubation period on production of α-Amylase

(iii) Impact of pH on production of α - Amylase : P^H 7.0 found to be favourable for maximum yield of α - Amylase (Fig. 9).

(iv) Influence of Temperature on production of α - Amylase: 30 °C found to enhance the highest yield of α - Amylase (Fig. 10).

(v) Influence of Carbon Sources on production of α - Amylase: Among different carbon sources tested Starch found to be the best carbon source

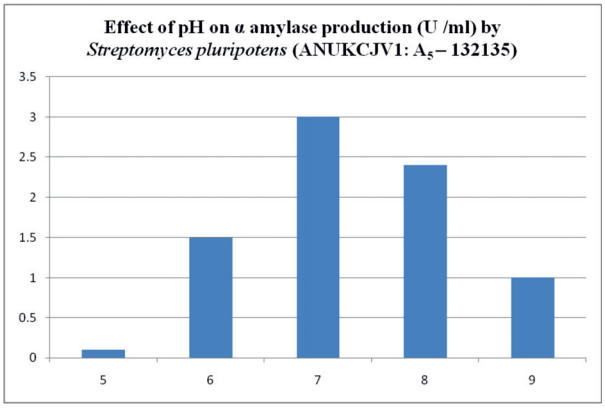


Fig. 9. Impact of pH on production of α - Amylase

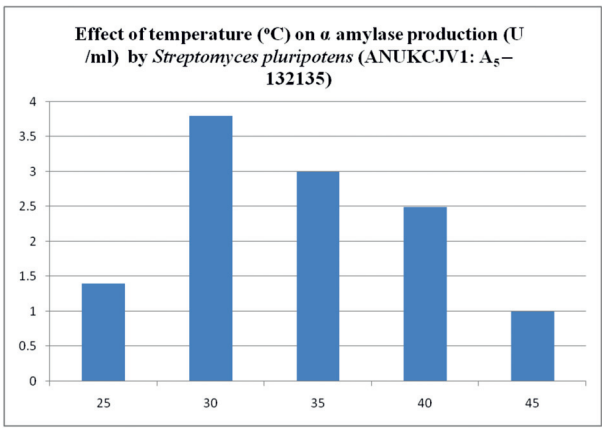


Fig. 10. Influence of Temperature on production of α - Amylase

with highest yield of α - Amylase (Fig. 11).

(vi) Influence of Nitrogen Sources on production of α - Amylase: With regard to Nitrogen sources, Beef extract found to be the best source of Nitrogen with maximum α - Amylase yield (Fig. 12).

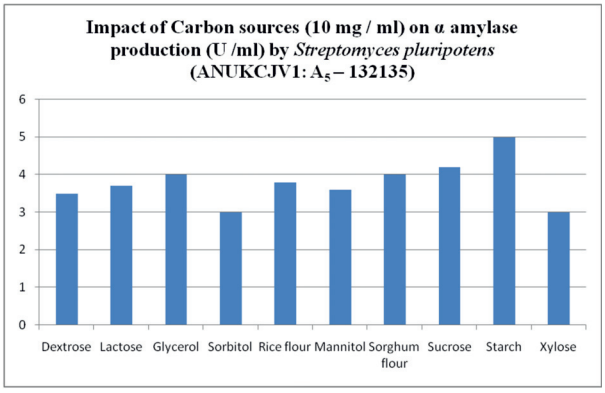


Fig. 11. Influence of Carbon Sources on production of α - Amylase

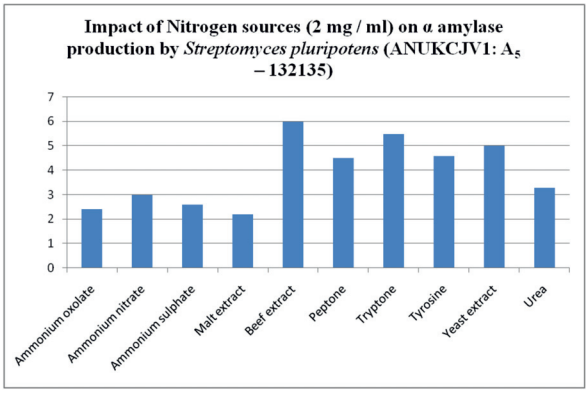


Fig. 12. Influence of Nitrogen Sources on production of α – Amylase

III. Optimization of experimental variables for α - Amylase enhanced production by *Streptomyces pluripotens* ANUKCJV1 (Potential Actinomycetes strain) by employing ISP 2 medium

(i) Influence of Incubation period on growth: Maximum Biomass growth was found at 96 hours of Incubation (Fig. 13).

(ii) Influence of Incubation period on production of α - Amylase: 96 hours of Incubation period reported maximum yield of α - Amylase (Fig. 14).

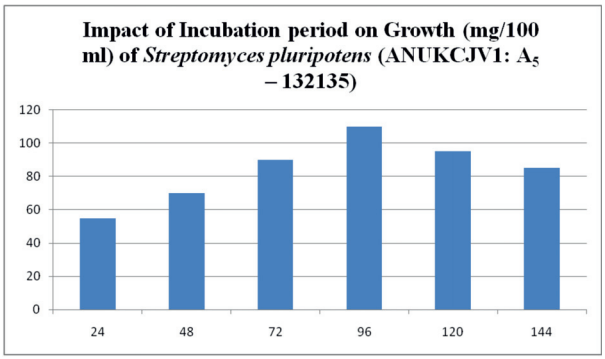


Fig. 13. Influence of Incubation period on growth

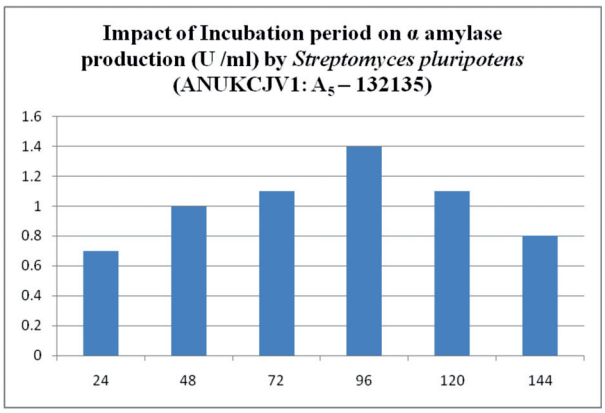


Fig. 14. Influence of Incubation period on production of α - Amylase

(iii) Impact of pH on production of α - Amylase: pH 8.0 reported to enhance maximum α - Amylase yield (Fig. 15).

(iv) Influence of Temperature on production of α - Amylase: Maximum α - Amylase yield reported at 30 ºC (Fig. 16).

(v) Influence of Carbon Sources on production of α - Amylase: Starch reported to be the best source of carbon with maximum α - Amylase yield (Fig. 17).

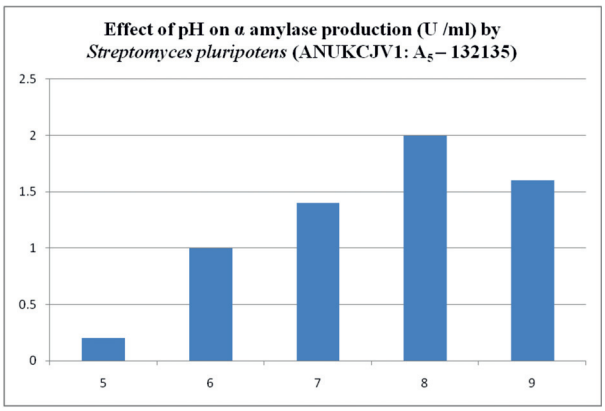


Fig. 15. Impact of pH on production of α - Amylase

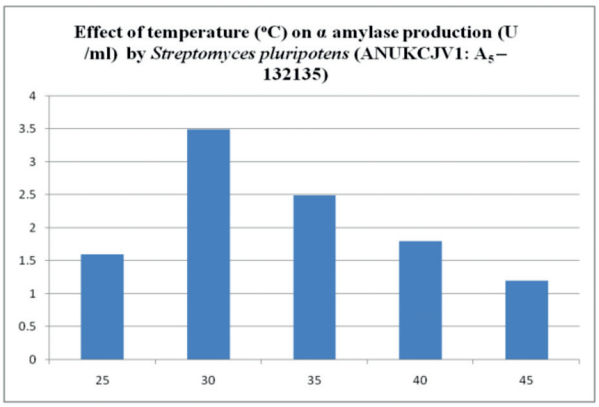


Fig. 16. Influence of Temperature on production of α - Amylase

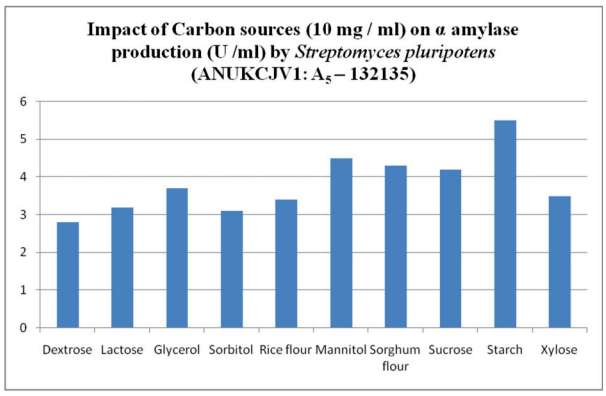


Fig. 17. Influence of Carbon Sources on production of α - Amylase

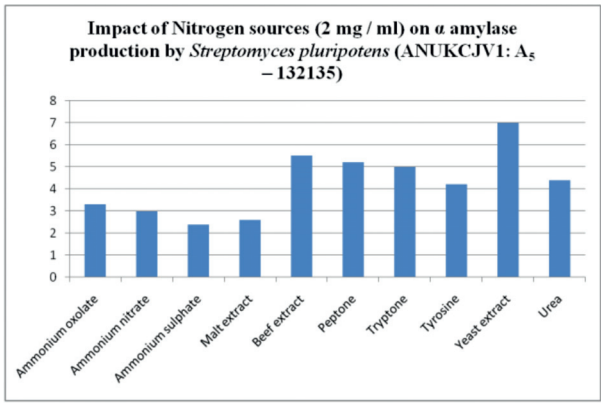


Fig. 18. Influence of Nitrogen Sources on production of α - Amylase

(vi) Influence of Nitrogen Sources on production of α-Amylase: Yeast extract enhances the highest yield of α - Amylase (Fig. 18).

IV. Comparative media analysis (ISP 4 Vs ISP 3 Vs ISP 2) based on Cumulative data of favourable variables of Optimization for construing of differential behaviour of α - Amylase bioactivity by the Potential Actinomycetes strain *Streptomyces pluripotens* ANUKCJV1

The Objective of the experiment was to decipher the differential α - Amylase behaviour based on cumulative data (Optimization) of comparative media analysis (ISP 4 Vs ISP 3 Vs ISP 2). Results suggests that ISP 4 found to be the favourable medium for α - Amylase production by the Potential Actinomycetes strain *Streptomyces pluripotens* ANUKCJV1 with cumulative value of 24.2 U/mL (with respect to favourable variables) where as the cumulative value of ISP 3 and ISP 2 media were 19.3 U/mL and 19.4 U/mL respectively (Fig. 19). The cumulative data of comparative optimization studies comprises of favourable experiment variables (Table 1).

V. Geographical Correlation

(i) Correlation between Geographical distance and the Number of Actinomycetes Strains

Totally 6 different geographical soil samples were

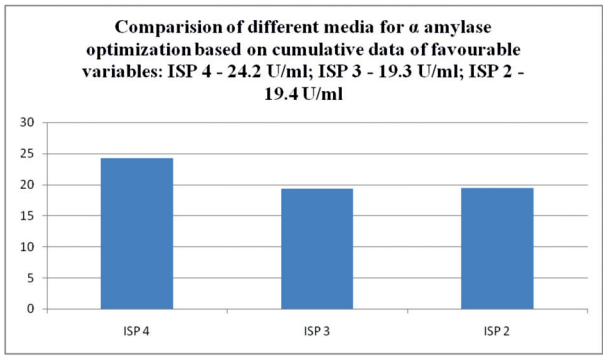


Fig. 19. Comparative media analysis

Table 1. Cumulative data of ISP 4, ISP 3 and ISP 2 media.

Optimization variable	ISP 4 (U/mL)	ISP 3 (U/mL)	ISP 2 (U/mL)
1. Incubation period	2.0	1.5	1.4
2. pH	4.0	3.0	2.0
3. Temeprature	4.2	3.8	3.5
4. Carbon source	6.0	5.0	5.5
5. Nitrogen source	8.0	6.0	7.0
Cumulative value	24.2 U/mL	19.3 U/mL	19.4 U/mL

collected from 6 different geographical locations: A_1 sample - collected from ~ 500 m from the shore; A_2 sample - collected from ~ 400 m from the shore; A_3 sample - collected from ~ 300 m from the shore; A_4 sample - collected from ~ 200 m from the shore; A_5 sample - collected from ~ 100 m from the shore; A_6 sample - collected from 0 m from the shore. Varied number of Actinomycetes strains reported from 6 different geographical soil samples: 1 strain of Actinomycetes each from A_3, A_4, A_5 and A_6 soil samples: A_3 - 1510118; A_4 - Ye; A_5 - 132135 and A_6 - 131044. 2 strains of Actinomycetes from A_2 and Geographical soil sample; A_2 - 130352; A_2 - 130305. 3 strains of Actinomycetes from A_1 Geographical soil sample; A_1 - 142838; A_1 - 143301; A_1 - 130236.

Results suggests that geographical correlation between the distance from the shore and number of strains of Actinomycetes was in tune of direct proportionality i.e. Greater the distance from the shore higher is the probability for isolation of strains of Actinomycetes as 3 strains of Actinomycetes collected from A_1 sample (~ 500 m from the shore), 2 strains from A_2 (~ 400 m from the shore) and 1 strain from A_3, A_4, A_5 and A_6 soil samples (Table 2). Geographical correlation depicted in Graph 1, which was a reverse 'L' shaped graph. Percentage wise share of different soil samples were depicted in Fig.66 which shows that A_1 sample occupies the major share of 33 % (3 strains) followed by A_2 - 22% (2 strains) and A_3 - 11%; A_4 - 11%; A_5- 11%; A_6- 11% (1 strain each). Pedological characteristics of different geographical soil samples were tabulated in Table 3.

(ii) Geographical Influence on the α - Amylase Bioactivity

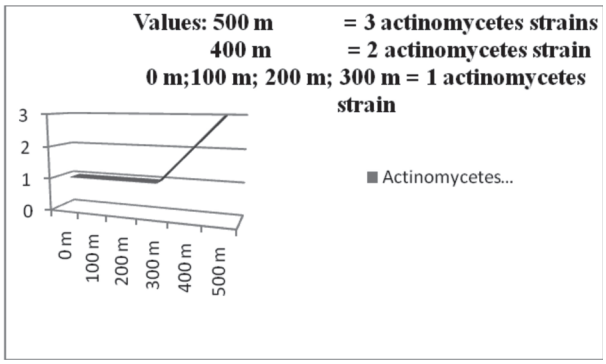
5 out of 9 Actinomycetes strains of varied geographical samples of soil exhibited α - Amylase bio-

Table 2. Number of strains of Actinomycetes isolated from different soil samples (A_1 to A_6)

S. No.	Sample	Number of Actinomycetes strains isolated		Geographical distance from the shore (In ascending order from the shore)
1	A_6	1	A_6 - 131044	0 m
2	A_5	1	A_5 - 132135	~ 100 m
3	A_4	1	A_4 - Ye	~ 200 m
4	A_3	1	A_3 - 1510118	~ 300 m
5	A_2		A_2 - 130352	
		2	A_2 - 130305	~ 400 m
			A_1 - 142838	
6	A_1	3	A_1 - 143301	~500 m
			A_1 - 130236	

Table 3. Pedological characteristics of different geographical samples of soil

S. No	Sample	Pedological characteristics
1	A_6	Alluvial: Ash grey colour with smooth texture
2	A_5	Alluvial: Ash grey colour with smooth texture
3	A_4	Alluvial: Ash grey colour with moderately rough texture
4	A_3	Alluvial: Light grey colour with moderately rough texture
5	A_2	Alluvial: Light grey colour with rough texture
6	A_1	Alluvial: Light grey colour with rough texture



Graph 1. Geographical Correlation

activity in the ascending order : A_2 - 130352 - 10 mm clearance zone; A_1 - 1301236 - 12 mm; A_1 - 142838 - 17mm; A_2 - 130305 - 21 mm and A_5 - 132135 - 30 mm respectively. Categorical wise geographical distribution (Fig. 20) depicts the point that major share of 80 % (40 % - A_1; 40 % - A_2) comes from distant geographical soil samples (A_1 and A_2) where as just 20 % comes from A_5 soil sample. Therefore, distant geographical soil samples ($A_1$and A_2) were better than geographical shore (A_6) and near shore (A_5 and A_4) soil samples for primary screening of α - Amylase. With respect to Secondary screening / production of α - Amylase, results suggests that 2 strains of Actinomycetes A5 - 132135 and A2 - 130305 reported as the potential strains for α - Amylase production. Whereas A_2 - 130352 was the least potential strain. Geographical correlation with the results was depicted in Graph 2. From the results it could be deciphered that, 2 geographical locations: A_5 (~ 100 m from the shore) and A_2 (~ 400 m from the shore) were the favourable zones for α - Amylase production.

Discussion

The objective of the comparative optimization study

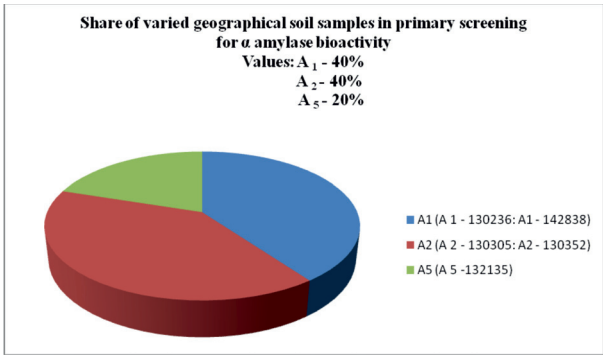
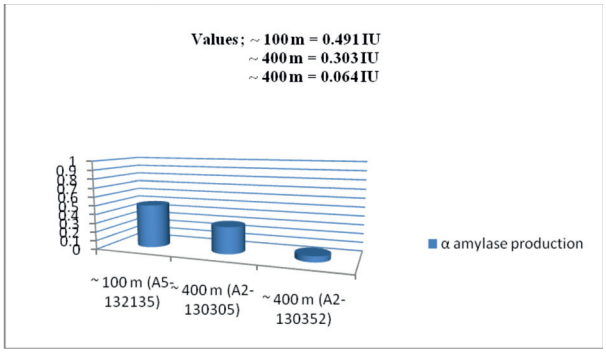


Fig. 20. Categorical wise geographical distribution

is to ascertain the favourable medium of the potential Actinomycetes strain *Streptomyces pluripotens*



Graph 2. Geographical correlation for production of α-Amylase

ANUKCJV1 which may pave the way for large scale exploration of α - Amylase which reportedly have huge industrial enzyme potentiality.

Optimization study of the potential Actinomycetes strain *Streptomyces pluripotens* ANUKCJV1 (A_5 - 132135) based on ISP 4 medium is in general consonance with the present knowledge. Initially optimization studies done by ISP 4 medium which suggests that under Incubation as optimization variable, highest yield of 2.0 U / mL was reported after 72 hours of Incubation period which is in consonance with Naragani *et al*. (2008) although differs in the amount of α - Amylase produced, *Streptomyces cheonanensis* VUK - A was the strain. Under the same line Yassein and Asfour (2012) suggested highest α - Amylase yield in *Streptomyces clavifer* after 72 hours. With regard to pH variable, our results got highest yield of α - Amylase of 4.0 U / mL at pH 7.0 which is line to Singh *et al*. (2012), they explored the strain *Streptomyces* sp. MSC 702. With regard to temperature variable, at 35°C highest yield of α - Amylase @ 4.2 U/mL was produced which contradicts with Narayana and Vijayalakshmi (2008) who reported 30 °C as the favourable variable with respect to *Streptomyces albidoflavus* where as Shaktimay and Ramesh (2008) suggested 50 °C as favourable variable in the strain *Streptomyces erumpens* MTCC 7317. Pertaining to Carbon sources, current results reported that Sorghum flour was the preferential source of carbon with the production of 6.0 U/mL which is in consonance with Kavitha and Vijayalakshmi (2010) who reported in the strain *Streptomyces tendae* TKVL_ 333. With respect to Nitrogen source our results reported that peptone was

the favourable source of Nitrogen @ 8.0 U / mL for the potential strain of Actinomycetes. Our results were in agreement with Kavitha and Vijayalakshmi (2010) who explored *Streptomyces tendae* TKVL_ 333 and suggested peptone as the preferential source of Nitrogen.

As discussed the optimization study of the potential strain further extended to comparative media analysis based on cumulative data (ISP 3 and ISP 2) besides ISP 4 medium to construe differential bioactive α - Amylase behaviour. With respect to ISP 3 medium the cumulative value of favourable variable was 19.3 U /mL, where as the cumulative value of favourable variables of ISP 2 medium was 19.4 U / mL respectively. Based on cumulative data it could be deciphered that ISP 4 medium with cumulative value of 24.2 U/mL found to be the favourable medium for *Streptomyces pluripotens* ANUKCJV1. Mangamuri *et al*. (2011) in order to accentuate the antibiotic potentiality of the rare Actinomycetes strain *Psuedonocardia* sp. VUK - 10 have conducted comparative media analysis for optimization studies and reported best medium found to be ISP 2 broth (amended yeast extract malt extract dextrose).

With respect to Geographical Influence on the number of Actinomycetes strains. Geographical corelation was in line to direct proportionality - Greater the distance from the shore higher is the possibility for isolating Actinomycetes strains. Pertaining to geographical influence on α - Amylase bioactivity. Both extreme (A_5 sample: ~ 100 m from the shore) and non extreme (A_2 sample: ~ 400 m from the shore) found to be favourable preferential zones for α - Amylase production. To the best of our knowledge ours was the first to conduct α - Amylase bioactivity under the lens of geographical influence.

Conclusion

From the comparative optimization study it could be concluded that ISP 4 medium found to be the favourable medium for *Streptomyces pluripotens* ANUKCJV1 with cumulative value of 24.2 U/mL, where as the cumulative value of ISP 3 was 19.3 U/mL and for ISP 2 was 19.4 U/mL. Therefore, large scale exploration of the strain by using ISP 4 medium may be done to tap the Industrial potentiality of α - Amylase. With regard to Geographical Influence; higher Actinomycetes strains were isolated from distant geographical soil samples, where as both extreme (A_5) and Non extreme (A_2) found to be favourable for α - Amylase production, which suggests that geography had an impact on the α - Amylase bioactivity of the Coringa Mangroves Actinomycetes.

Conflicts of Interests: None Declared.

Acknowledgements

SK Chand Basha is immensely thankful to his Ph.D. Guide Prof.K.R.S. Sambasiva Rao and H.O.D. of Zoology and Aquaculture, A.N.U. for timely help, to U.G.C. for awarding the B.S.R fellowship, to Dept. of Life Sciences, CHRIST (Deemed to be University) for timely support, to his parents for sustaining a conducive academic atmosphere and finally to Almighty for his blessings.

References

Chand Basha, S.K. and Sambasiva Rao, K.R.S. 2017. An Intrinsic Assessment of Bioactive Potentiality of Mangroves Actinomycetes. *Journal of Entomology and Zoology Studies.* 5(1) : 20-26.

Jicheng Yu, Liu Zhang, Qiu Liu[1], Xiaohui Qi, Ying Ji and Beom Seok Kim, 2015. Isolation and characterization of actinobacteria from Yalujiang coastal wetland, North China. *Asian Pac J Trop Biomed*. 5 (7) : 555–560

Krishna Naragani, Vijayalakshmi Muvva, Rajesh Kumar Munaganti and Hima Bindu, B.S. S.N. 2015. Studies on Optimization of Amylase Production by *Streptomyces cheonanensis* VUK-A Isolated from Mangrove Habitats. *Journal of Advances in Biology & Biotechnology.* 3 (4) : 165-172.

Kavitha, A. and Vijayalakshmi, M. 2010. Production of amylases by *Streptomyces tendae* TKVL_ 333. *Int. J. Cur. Res*. 10 : 110–114.

Mangamuri Usha Kiranmayi, Poda Sudhakar, Kamma Sreenivasulu and Muvva Vijayalakshmi, 2011. Optimization of Culturing Conditions for Improved Production of Bioactive Metabolites by *Pseudonocardia* sp. VUK-10. *Mycobiology*. 39 (3) : 174-181.

Narayana, K.J.P. and Vijayalakshmi M. 2008. Production of extracelluar α-amylase by *Streptomyces albidoflavus*. *Asian J. Biochem.* 3 : 194–197.

Ramesh, S., Rajesh, M. and Mathivanan, N. 2009. Characterization of a thermostable alkaline protease produced by marine *Streptomyces fungicidicus* MML1614. *Bioprocess Biosyst Eng*. 32 (6) : 791-800.

Selvam, K., Vishnupriya, B. and V. Subhash Chandra Bose, 2011. Screening and Quantification of Marine Actinomycetes Producing Industrial Enzymes Amylase,

Cellulase and Lipase from South Coast of India. *International Journal of Pharmaceutical & Biological Archives.* 2 (5) : 1481-1487.

Chand Basha, S.K., Sambasiva Rao, K.R.S., Venkatanagaraju, E. and Jobi Xavier, F.R. 2019. An evaluation of the geographical influence on the α-amylase bioactivity of actinomycetes –isolated from Coringa Mangroves. *Journal of Pharmacognosy and Phytochemistry.* 8 (1) : 775-783.

Shaktimay Kar and Ramesh Chandra Ray, 2008. Partial characterization and optimization of extracellular thermostable Ca^{2+} inhibited α amylase production by *Streptomyces erumpens* MTCC 7317. *J. Sci. Ind. Res*. 67: 58–64.

Valli, S., Suvathi Sugasini, S., Aysha, O.S., Nirmala, P., Vinoth Kumar, P. and Reena, A. 2012. Antimicrobial potential of Actinomycetes species isolated from marine environment. *Asian Pac J Trop Biomed.* 2 (6) : 469-473.

Yassien, M.A.M. and Asfour, H.Z. 2012. Improved production, purification and some properties of ∞- amylase from Streptomyces clavifer. *Afr. J. Biotechnol.* 11 : 14603–14611.