ISSN: 2583-7354

International Journal of Emerging Knowledge Studies

Publisher's Home Page: https://www.ijeks.com/

Fully Open Access Research Paper

Isolation and Identification of Amylase Producing Bacteria from Mangrove Sediment, Vellar Estuary, Parangipettai.

P. Balamurugan[1], S. Harikrishnan*[2], S. Sathishkumar [3], U. Arunsuthish[2], M. Syed Abdulrahman [2], U. Sakthivel [1], P. Anantharaman [2]

[1]Department of Microbiology, A.V.C. College (Autonomous), Mannampandal.

[2]Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai.

[3]Department of Biotechnology, Bharathidasan University, Tiruchirapalli.

*Corresponding Author: hariugin97@gmail.com

Article Info: - Received : 02 March 2023 **Accepted** : 12 March 2023 **Published** : 25 March 2023

Abstract

In the present study, Mangrove sediment sample from vellar estuary were collected and aerobic forms of bacteria were isolated from vellar estuary, Parangipettai. The TVC (Total Viable Count) of Mangrove sediment sample were ranged from $1.1x10^3$ to $2.5x10^6$ CFU/g. In the present work, a total of 05 different bacterial species were detected in the sample. Totally five bacterial isolates viz., AVC 01, AVC 02, AVC 03, AVC 04 and AVC 05 were selected by colour, shape and morphologically for amylase enzyme activity. Among the total of 05 bacteria isolated from the Mangrove sediment sample, the isolate designated as AVC 05 which showed highest zone of clearance on starch agar plate was selected and used for further optimization and amylase production. The most potential Strain (AVC 05) was identified as *Mesobacillus subterraneus* using biochemical methods as well as 16S r-RNA sequencing methods. The sequence was submitted to NCBI and the Gen Bank accession number is *SUB13018627 Seq1* OQ725912. The amylase enzyme producing potential Strain AVC 05 *Mesobacillus subterraneus* was further optimized for different physicochemical growth parameters using broth medium. Various parameters on growth of *Mesobacillus subterraneus* optimum conditions observed for the maximum biomass and potential growth Incubation 36 hrs (2.22 OD value at 600 nm), Agitation 160 (1.72 OD value at 600 nm), pH 8 (1.73 OD value at 600 nm), Temperature- 37° (1.75 OD value at 600 nm), Salinity 2.5 PSU (1.81 OD value at 600 nm), Carbon source glucose (1.36 OD value at 600 nm), Concentration of glucose (%) 2 (1.98 OD value at 600 nm), Effect of nitrogen source (1%), Yeast extract (1.98 OD value at 600 nm), Effect of concentration of yeast extract (%) 1.5 (1.93 OD value at 600 nm). Growth in the mass cultivation medium under optimized conditions at the end of 36 h of incubation was 3.42 OD with the amylase enzyme activity zone of 1.9 mm.

Keywords: *Mangrove Sediment, Amylase Enzyme, Mesobacillus Subterraneus, Optimization.*

1. INTRODUCTION

A marine enzyme is a unique protein molecule with novel properties derived from an organism whose natural habitat comprises saline or brackish water. Marine microorganisms have been attracting more and more attention as a resource for new enzymes, because the microbial enzymes are relatively more stable and active than the corresponding enzymes derived from plants or animals **(Kin, 2006)**. In recent years, researchers have isolated a variety of enzymes with special activities from marine bacteria, actinomycetes, fungi and other marine microorganisms, and some products have already been used in industrial applications **(Haefiner*et al.,* 2003 and Ghosh *et al.,* 2005)**.

Total protease sales represent more than 60% of all industrial enzyme sales in the world. In 1960, Dane first isolated alkaline protease from *Bacillus licheniformis*. So far, it is still found that microorganisms are the most suitable resources for protease production. In 1972, Nobou Kato isolated a new type of alkaline protease from marine *Psychrobacter*, and since then quite a few proteases have been continually obtained from marine microorganisms. An alkaline protease, previously isolated from a symbiotic bacterium found in the Gland of Deshayes of a marine shipworm, was evaluated as a cleansing additive.

Amylases are the enzymes that most widely used enzymes in industries. That catalyses the hydrolysis of starch into commercial production of sugar syrups which consist of glucose, maltose and higher oligosaccharides **(Hagihara*et al.*, 2001)**. Amylase present in the saliva of human and some other mammals for chemical process of digestion. Foods that contain great amount of starch but slight amount of sugar such as rice and potatoes, it may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into sugar. Amylases are a group of enzymes which are mainly used in starch processing industries for hydrolysis of starch into simple sugars **(Akpan*et al.*, 1999)**.

The microbial amylases meet up industrial demands, a large number of them are offered commercially and they have almost completely replaced chemical hydrolysis of starch processing industry. Both α-amylases and β-amylase are imperative in brewing beer and liquor from sugars derived from starch. Amylases are used in bread making and to break down complex sugars, such as starch into simple sugars. In fermentation, yeast feeds on these simple sugars and converts into waste product of alcohol and CO_2. In the present study, the amylase enzyme production was found to be growth dependent.

2. METHODOLOGY

2.1 Collection of sediment samples

Mangrove sediment samples were collected in sterile containers using a clean sterile PVC (25 cm height with 5 cm diameter) corer after rinsing its inner surface with 95% alcohol. Samples were taken from 15 to 20 cm depth in velar estuary Parangipettai, South east coast India. Samples were transferred to laboratory immediately and analysed for microbial groups within 2- 4 hrs of sampling.

2.2 Isolation and enumeration of Total Heterotrophic Bacteria (THB) from marine Sediment samples

For the isolation of Total Heterotrophic Bacteria (THB) from marine sediments, 1g ram of sediment sample was aseptically weighed and transferred to a sterile conical flask containing 99 ml of sterile 50% aged seawater which was used as a diluent. Samples were serially diluted up to 10-5 with sterilized 50% aged seawater. 0.1ml of serially diluted sample was plated using spread plate technique on Zobell marine agar medium **(Zobell, 1941)** (Himedia, Mumbai) for analyzing total heterotrophic bacteria (THB). Plates were incubated at 28±2 ºC for 24 to 48 hrs. The microbial load (density) in the given sample was calculated using the formula given below and it was expressed as Colony Forming Units (CFU) per gram of the sample. Each Morphologically different colony was isolated and streaked on Zobel marine agar slants and were maintained at 4ºC. Total microbial load in the given sample ($CFU.g^{-1}$) = Total number of colonies/ Total Volume of the sample x Volume of sample plated (0.1 ml) x dilution factor.

2.3 Preparation of bacterial inoculum

Bacterial inoculum was prepared according to CLSI, 2007 (Clinical and Laboratory Standards Institute) by inoculating a loopful of test organisms in 5ml of nutrient broth and incubated at 37ºC for 3-5 hrs till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards and then used for the determination of amylase enzyme activity.

2.4 Amylase assay

Hence in this study amylase production alone tried with strains AVC 01- AVC 05. Bacterial cell free supernatant was put in the well. Plated on starch agar plates (Hi Media, Mumbai, India) using spread plate

method. Plates were incubated at room temperature (28±2℃) for 3-7 days. After incubation, plates were added with 1% of iodine solution for 5 min. and washed with water to remove the excess stain (Bahadureet *al.,* 2010). Formation of clear zone around the colonies indicates positive reaction for amylase production. Based on morphology, individual bacterial strains were collected and secondary screening in starch agar was done using well assay. Based on the highest zone of clearance, the most potential strain was selected, stored on starch agar slant and used for further study.

2.5 Identification of the most potential strain

The most potential strain was identified based on morphological, cultural, biochemical and physiological characteristics according to the Bergey's Manual of Determinative Bacteriology (Buchanan *et al.,* 1974) and also using 16S r-RNA sequencing was done using standard procedure.

2.6 Optimization of growth of potential strain

Several physiochemical parameters like incubation period, temperature, agitation, PH, salinity, carbon and nitrogen sources, on growth were optimized parameters were used for the further subsequent optimized steps.

2.7 Mass scale culture

The optimized growth conditions such as 36 hrs of incubation, agitation -160 rpm, pH-7, temperature 37℃, and salinity 2.5 PSU, 2.0% glucose as the carbon source and 1.5% yeast extract as the nitrogen source were maintained in the medium. Mass scale culture was done in 2L conical flasks with 1.0 L of medium. Growth and antimicrobial activity were evaluated at the end of 36 hrs incubation. Zone of clearance on agar plate using agar well diffusion method was considered as the criterion for antimicrobial activity and it was done as previously mentioned in the screening step as described by Millette *et al.,* 2007. Trial runs were done up to 48 hrs to ascertain the optimum period.

3. RESULT AND DISCUSSION

3.1 Isolation of microbes and Microbial density

In the present study, ten marine sediment samples collected from Mangrove sediment sample, vellar estuary (Fig.4 & 5) were plated on Zobell marine agar and the observed microbial density was from $1.1x10^3$ to $2.5x10^6$ CFU/g in the sediment sample. Totally five bacterial isolates viz., AVC 01, AVC 02, AVC 03, AVC 04 and AVC 05 were selected by colour, shape and morphologically for further study.

3.2 Amylase production

Among the total number, 05 bacteria isolated from the Mangrove sediment sample, the isolate designated as AVC 05 which showed highest zone of clearance on starch agar plate was selected and used for further optimization and amylase production.

3.3 Identification of the potential strain

The most potential Strain (AVC 05) was identified as *Mesobacillus subterraneus* using biochemical methods as well as 16S r-RNA sequencing methods. The sequence was submitted to NCBI and the Gen Bank accession number is *SUB13018627 Seq1* OQ725912 and the sequence is given Fig. 3. The phylogenetic tree was constructed using partial 16S r-RNA gene sequences using Neighbor joining method **(**Table. 1). Al-Thubiani *et al.,* 2018 identified antimicrobial peptide producing *B. megatarium* species using 16S r-RNA sequence analysis. Similarly, Usta *et al.,* 2018 identified the antimicrobial compound producing *Brevibacillus laterosporus* Strain EA62 using 16S r-RNA analysis.

3.4 Optimization of growth and amylase production

Optimum conditions play a key role in any fermentation process to achieve the maximum growth and production of microbial products including enzyme in the present case. Optimum biosynthesis varied among different enzymes to different substrates also (Smith *et al.,* 1996). In the present work, the other optimum conditions observed for the maximum biomass and potential growth Incubation 36 hrs (2.22 OD value at 600 nm, Agitation 160 (1.72 OD value at 600 nm), pH 8 (1.73 OD value at 600 nm), Temperature- 37° (1.75 OD value at 600 nm), Salinity 2.5 PSU (1.81 OD value at 600 nm), Carbon source glucose (1.36 OD value at 600 nm), Concentration of glucose 2 % (1.98 OD value at 600 nm), Effect of nitrogen source (1%) Yeast extract (1.98 OD value at 600 nm), Effect of concentration of yeast extract (%) 1.5 (1.93 OD value at 600 nm)

Inoculum concentration is considered to be an important factor in enzyme activity which enables the strains substrate utilization ability. The results of the present study are in agreement with Sharma *et al.,* 1996 and Shah *et al.,* 2014 who found decreased growth with the increased inoculum size beyond the optimum concentration. The smaller inoculum size

extended the lag phase and support only growth to certain extent then the growth was found to be reduced. It is also true with higher concentration of inoculums.

Sundarapandiyan and Jayalakshmi (2017), Udayadevi et al.,2023 reported maximum amylase production 64 U/ml/min from a marine *Bacillus subtilis* SJ33 isolated from Vellar estuary at pH 7, 35°C, 2% salinity, starch and peptone as the carbon and nitrogen sources respectively under submerged fermentation after 48 hrs of incubation

3.5 Mass cultivation and growth of *Mesobacillus subterraneus* under optimum conditions in shake flask culture

The optimized growth conditions such as 36 hrs of incubation, agitation -160 rpm, pH-7, temperature 37°C, and salinity 2.5%, 2.0% glucose as the carbon source and 1.5% yeast extract as the nitrogen source were maintained in the 2L shake flask containing 1.0 L of medium. Growth and antimicrobial activity were evaluated at the end of 36 hrs incubation. The results showed the growth 3.42 OD with the antimicrobial activity zone of 3.9 mm under optimized conditions at the end of 36 h of incubation. (Fig. 4)

4. CONCLUSION

Hence, the *Mesobacillus subterraneus* isolated from the Mangrove sediment sample, vellar estuary was a potential candidate for the production of amylase enzyme. There are many potential and widely used applications of this enzyme on the industrial front. Enzymes have replaced the previously used chemical methods of hydrolysis in various industrial sectors to make the process environment friendly and make processes easier.

REFERENCES

Azam, M., Bhatt, V. (2015). Like father, like son? intergenerational educational mobility in india. Demography 52(6):1929–1959

Felipe González Castro, Joshua G. Kellison, Stephen J. Boyd and Albert Kopak. A Methodology for Conducting Integrative Mixed Methods Research and Data Analyses.Journal of Mixed Methods Research 2010 4: 342.DOI: 10.1177/1558689810382916

Reeta Rani Singhania, Rajeev K.Sukumaran, Anil Kumar Patel , Christian Larroche , Ashok Pandey.Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. doi:10.1016/j.enzmictec.2010.03.010

Ashok Pandey, Poonam Nigam, Carlos R. Soccol, Vanete T. Advances in microbial amylases. Soccol, Dalel Singh and Radjiskumar Mohan.DOI: 10.1042/BA19990073

Ahlawat, S., Dhiman, S. S., Battan, B., Mandhan, R. P., & Sharma, J. (2009). Pectinase production by Bacillus subtilis and its potential application in biopreparation of cotton and micropoly fabric. *Process Biochemistry*, *44*(5), 521-526.

Ahmadi-Javid, A. (2012) Entropic Value-At Risk: A New Coherent Risk Measure. Journal of Optimization: Theory and Applications, 155, 1105-1123.https://doi.org/10.1007/s10957-011-9968-2

Akpan, I., Bankole, M.O. & Adesemowo, A.M. A rapid plate culture method for screening of amylase producing micro-organisms. Biotechnology Techniques 13, 411–413 (1999). https://doi.org/10.1023/A:1008965808641

Alexandra Abate, Sarah Bridle, Luis F. A. Teodoro, Michael S. Warren, Martin Hendry, Peculiar velocities into the next generation: cosmological parameters from large surveys without bias from non-linear structure, Monthly Notices of the Royal Astronomical Society, Volume 389, Issue 4, October 2008, Pages 1739–1749, https://doi.org/10.1111/j.13652966.2008.13637.

Al-Thubiani, A. S., Maher, Y. A., Fathi, A., Abourehab, M. A., Alarjah, M., Khan, M. S., & Al-Ghamdi, S. B. (2018). Identification and characterization of a novel antimicrobial peptide compound produced by *Bacillus megaterium* strain isolated from oral microflora. *Saudi pharmaceutical journal*, *26*(8), 1089-1097.

Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev. 1995 Mar;59(1):143-69. doi: 10.1128/mr.59.1.143-169.1995. PMID: 7535888; PMCID: PMC239358.

Anna Stief, Simone Altmann, Karen Hoffmann, Bikram Datt Pant, Wolf-Rüdiger Scheible and Isabel Bäurle. Arabidopsis miR156 Regulates Tolerance to Recurring Environmental Stress through SPL Transcription Factors. Plant Cell 2014;26;1792-1807; originally published online April 25, 2014;DOI 10.1105/tpc.114.123851

Bakri SJ, Pulido JS, McCannel CA, Hodge DO, Diehl N, Hillemeier J. Immediate intraocular pressure changes following intravitreal injections of triamcinolone, pegaptanib, and bevacizumab. Eye (Lond). 2009 Jan;23(1):181-5. doi: 10.1038/sj.eye.6702938. Epub 2007 Aug 10. PMID: 17693999.

Ballschmiter, M., Futterer, O., & Liebl, W. (2006). Identification and characterization of a novel intracellular alkaline α-amylase from the hyperthermophilic bacterium Thermotoga maritima MSB8. *Applied and environmental microbiology*, *72*(3), 2206-2211.

Subash C. B. Gopinath,Periasamy Anbu, M. K. Md Arshad, Thangavel Lakshmipriya,Chun Hong Voon,Uda Hashim,and Suresh V. Chinni. Biotechnological

Processes in Microbial Amylase Production. https://doi.org/10.1155/2017/1272193

Das, P., Mukherjee, S., & Sen, R. (2009). Biosurfactant of marine origin exhibiting heavy metal remediation properties. *Bioresource technology*, *100*(20), 4887-4890.

DAVID MALAKOFF.Extinction on the High Seas.DOI: 10.1126/science.277.5325.486

Hiroshi Hagihara,Yasuhiro Hayashi, Keiji Endo, Kazuaki Igarashi, Tadahiro Ozawa, Shuji Kawai, Katsuya Ozaki, Susumu Ito. Deduced amino-acid sequence of a calcium-free α-amylase from a strain of Bacillus. https://doi.org/10.1046/j.1432-1327.2001.02308.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular biology and evolution*, *29*(8), 1969-1973.

Erdal, S., Aydın, M., Genisel, M., Taspınar, M. S., Dumlupinar, R., Kaya, O., & Gorcek, Z. (2011). Effects of salicylic acid on wheat salt sensitivity. *African Journal of Biotechnology*, *10*(30), 5713-5718.

Foster KR, Wenseleers T, Ratnieks FL. Kin selection is the key to altruism. Trends Ecol Evol. 2006 Feb;21(2):57-60. doi: 10.1016/j.tree.2005.11.020. Epub 2005 Dec 20. PMID: 16701471

José Manuel Pueyo Rodrigues de Carvalho, From discrete biofilm model formation to queuing transient analysis. Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa.

Frost, G.M. and Moss, D.A. (1987) Production of Enzymes by Fermentation. In: Rehm, H.J., Reed, G., Eds., Biotechnology, Vol. 7a, VCH, Weinheim, 65-102.

G. Muralikrishna, M. Nirmala.Cereal α-amylases—An overview.https://doi.org/10.1016/j.carbpol.2004.12.002.

Gangadharan, D., Sivaramakrishnan, S., Nampoothiri, K. M., & Pandey, A. (2006). Solid culturing of Bacillus amyloliquefaciens for alpha amylase production. *Food Technology & Biotechnology*, *44*(2).

Ghosh K, et al. (2005) Spectroscopic and biochemical characterization of heme binding to yeast Dap1p and mouse PGRMC1p. Biochemistry 44(50):16729-36

Gusakov, A. V., Kondratyeva, E. G., & Sinitsyn, A. P. (2011). Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. *International journal of analytical chemistry*, *2011*.

Hall-Stoodley, L. & Stoodley, P. Evolving concepts in biofilm infections. Cell Microbiol. 11: 1034-43

Hmidet, N., Ali, N. E. H., Haddar, A., Kanoun, S., Alya, S. K., & Nasri, M. (2009). Alkaline proteases and thermostable α-amylase co-produced by Bacillus licheniformis NH1: Characterization and potential application as detergent additive. *Biochemical Engineering Journal*, *47*(1-3), 71-79.

Fariha Hasan , Aamer Ali Shah, Abdul Hameed. Industrial applications of microbial lipases.doi:10.1016/j.enzmictec.2005.10.016

Vineeta Singh, Shafiul Haque, Harshita Singh1, Jyoti Verma, Kumari Vibha1,Rajbir Singh, Arshad Jawed and C. K. M. Tripathi . Isolation, Screening, and Identification of Novel Isolates of Actinomycetes from India for Antimicrobial Applications.doi: 10.3389/fmicb.2016.01921

Jha, R.K.; Zi-rong, X. Biomedical Compounds from Marine organisms. Mar. Drugs 2004, 2, 123-146. https://doi.org/10.3390/md203123

Kalra, M. K., & Sandhu, D. K. (1986). Optimal production of cellulolytic enzymes and their location in Trichoderma pseudokonigii. *Acta Biotechnologica*, *6*(2), 161-166.

Kilic U, Kilic E, Reiter RJ, Bassetti CL, Hermann DM. Signal transduction pathways involved in melatonin-induced neuroprotection after focal cerebral ischemia in mice. J Pineal Res. 2005 Jan;38(1):67-71. doi: 10.1111/j.1600-079X.2004.00178.x. PMID: 15617539.

Kirk, O., Borchert, T. V., & Fuglsang, C. C. (2002). Industrial enzyme applications. *Current opinion in biotechnology*, *13*(4), 345-351.

Kulp, K., Ponte Jr, J. G., & D'Appolonia, B. L. (1981). Staling of white pan bread: fundamental causes. *Critical Reviews in Food Science & Nutrition*, *15*(1), 1-48.

Kundu, A. K., & Das, S. (1970). Production of amylase in liquid culture by a strain of Aspergillus oryzae. *Applied microbiology*, *19*(4), 598-603.

Levine, N. D. (1975). Buchanan, RE & Gibbons, NE, eds. 1974. Bergey's Manual of Determinative Bacteriology. Williams & Wilkins Co., Baltimore, Md. 21202. xxvi+ 1246 pp. $45.00.

Marine fungi.Amy S. Gladfelter, Timothy Y. James, and Anthony S. Amend.Current Biology 29, R183–R199, March 18, 2019.DOI: 10.1016/j.cub.2019.02.009

Melanie Bergman Alan C. TaylorMoore.Physiological stress in decapod crustaceans (Munida rugosa and Liocarcinus depurator) discarded in the Clyde Sea Norway lobster fishery January 2001Journal of Experimental Marine Biology and Ecology 259:215-229

Millette, M. C. L. T., Le Tien, C., Smoragiewicz, W., & Lacroix, M. (2007). Inhibition of Staphylococcus aureus on beef by nisin-containing modified alginate films and beads. *Food control*, *18*(7), 878-884

Miranda OA, Salgueiro AA, Pimentel MCB et al (1999) Lipase production by a Brazilian strain of Penicillium citrinum using an industrial residue. Bioresour Technol 69:145–147

Mitidieri, S., Martinelli, A. H. S., Schrank, A., & Vainstein, M. H. (2006). Enzymatic detergent formulation containing amylase from Aspergillus niger: a comparative study with commercial detergent formulations. *Bioresource technology*, *97*(10), 1217-1224.

N. Saroja , T.R. Shamala , R.N. Tharanathan.Biodegradation of starch-g-polyacrylonitrile, a packaging

material,by Bacillus cereus.DOI:10.1016/S0032-9592(00)00190-4.

Nouadri, T., Meraihi, Z., Shahrazed, D. D., & Leila, B. (2010). Purification and characterization of the α-amylase isolated from *Penicillium camemberti* PL21. *Afr. J. Biochem. Res*, *4*(6), 155-162.

Omemu, A. M., Akpan, I., Bankole, M. O., & Teniola, O. D. (2005). Hydrolysis of raw tuber starches by amylase of *Aspergillus niger* AM07 isolated from the soil. *African Journal of Biotechnology*, *4*(1), 19-25.

Paquet, V. E. R. O. N. I. Q. U. E., Croux, C. H. R. I. S. T. I. A. N., Goma, G. E. R. A. R. D., & Soucaille, P. H. I. L. I. P. P. E. (1991). Purification and characterization of the extracellular alpha-amylase from Clostridium acetobutylicum ATCC 824. *Applied and Environmental Microbiology*, *57*(1), 212-218.

Aleena Sumrin, Waqar Ahmad, Bushra Ijaz, Muhammad Tahir Sarwar, Sana Gull, Humera Kausar, Imran Shahid, Shah Jahan, Sultan Asad, Mureed Hussain and Sheikh Riazuddin. Purification and medium optimization of α-amylase from Bacillus subtilis 168 .DOI: 10.5897/AJB10.1966

Rhee, J., Park, T. and Lee, D.H. (2010) Drivers of Innovativeness and Performance for Innovative SMEs in South Korea: Mediation of Learning Orientation. Technovation, 30, 65-75.http://dx.doi.org/10.1016/j.technovation.2009.04.008

Sajedi RH, Naderi-Manesh H, Khajeh KH, Ranjbar B, Ghaemi N,Naderi-Manesh M (2004). Purification, characterization, and structural investigation of a new moderately thermophilic and partially calcium-independent extracellular alpha-amylase from Bacillus sp. TM1. Appl Biochem Biotechnol. 119: 41-50.

Seyyed Mahmoud Hashemi, Reza Rostami, Mohammad Kazem Hashemi & ChristosA. Damalas (2012): Pesticide Use and Risk Perceptions among Farmers in Southwest Iran, Human and Ecological Risk Assessment: An International Journal, 18:2, 456-47

Shafique, S., Bajwa, R., & Shafique, S. (2009). Screening of Aspergillus niger and A. flavus strains for extra cellular alpha-amylase activity. *Pak. J. Bot*, *41*(2), 897-905.

Shah, I. J., Gami, P. N., Shukla, R. M., & Acharya, D. K. (2014). Optimization for α-amylase production by Aspergillus oryzae using submerged fermentation technology. *Basic Research Journal of Microbiology*, *1*(4), 01-10.

Shirley A. Pomponi .The bioprocess-technological potential of the sea.https://doi.org/10.1016/S0079-6352(99)80092-7

Smith BJ, Bauman AE, Bull FC, Booth ML, Harris MF. Promoting physical activity in general practice: a controlled trial of written advice and information materials. Br J Sports Med. 2000 Aug;34(4):262-7. doi: 10.1136/bjsm.34.4.262. PMID: 10953898; PMCID: PMC1724212.

Sundarapandiyan, B., & Jayalakshmi, S. (2017). Isolation, Screening an d Optimization of Amylase Production by a Marine Bacterium Bacillus Subtili s SJ33. *Intl J Adv Res Bio Sci*, *4*, 8-14.

Walker, M. F. et al. (2017) Improving the development, monitoring and reporting of stroke rehabilitation research: Consensus-based core recommendations from the Stroke Recovery and Rehabilitation Roundtable. International Journal of Stroke, 12(5), pp. 472-479.(doi:10.1177/1747493017711815)

Mamta Kumari and Jagdeep Singh. WATER CONSERVATION: STRATEGIES AND SOLUTIONS.

Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. Proc Natl Acad Sci U S A. 1998 Jun 9;95(12):6578-83. doi: 10.1073/pnas.95.12.6578. PMID: 9618454; PMCID: PMC33863.

Zia-UL-Haq, M., Shahid, S. A., Khan, B. A., Imran, I., Qayum, M., Akhter, M., ... & Muhammad, S. (2012). Nematicidal potential of selected flora of Pakistan. *J. Med. Plants Res*, *6*(24), 4087-4090.

Cite this article as: P.Balamurugan et al. (2023). Isolation and Identification of Amylase Producing Bacteria from Mangrove Sediment, Vellar Estuary, Parangipettai. International Journal of Emerging Knowledge Studies. 2(3), pp. 52-57.